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BIOLOGICAL ASPECTS OF CANCER

BY JULIAN HUXLEY
Evolution: The Modern Synthesis

EDITED BY JULIAN HUXLEY, A. C. HARDY, E. B. FORD
Evolution as a Process

JULIAN HUXLEY
F.R.S.

Biological Aspects of Cancer

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PREFACE

SOME justification is needed for the publication of a book on cancer by one who has never done original work on the subject, or indeed in any field of medical science. The primary responsibility rests on Dr. Rhoads of the Sloan-Kettering Foundation for Cancer Research in New York, who in 1955 invited me to deliver the first Alfred P. Sloan Lecture under the auspices of the Institute, on some aspect of the cancer problem. I felt that my lack of any detailed knowledge of the subject precluded my acceptance of this honourable assignment: but when he countered by saying that a fresh and outside view was just what the Institute wanted, the ground was cut from under my feet, and with some trepidation I agreed.

In the course of preparing the lecture I came across so many unfamiliar facts and was driven to so many ideas novel to me as a biologist that I felt it would be worth while to publish them in a more comprehensive and systematic form, and accordingly asked and was given permission to work up my material into a review for *Biological Reviews,* which demanded another year's intensive work. As time went on and I discovered more and more facts and ideas to digest, my manuscript came to exceed the limit allowed for articles in that journal, and the Editor, Professor H. M. Fox, F.R.S., kindly agreed that it should be published in the form of two separate reviews, which eventually appeared in the last issue of vol. 31 (p. 474) and the first issue of vol. 32 (p. 1).

Finally, I had such numerous requests for reprints, and so many suggestions from workers in various disciplines that the material should be reissued in book form, that I obtained the consent of Professor Fox to this course. In the present volume the two reviews have been combined, and considerable new material added.

I can truthfully say that the preparation of these two reviews involved me in more hard labour than anything I have attempted since I took my Final Honours examination in Zoology at Oxford, more years ago than I care to remember. I could never have finished them if I had been occupied with teaching, administration, or the need to produce a stream of research papers. Indeed it may be a good thing to have a few elderly scientists quit of all such professional obligations and free to devote themselves now and again to general surveys.

But labour alone is not sufficient excuse for inflicting another book on the world. My excuse is that in the course of amassing and digesting the material I came to a vivid realization of the fact that cancer, far from being a field of purely medical concern, is a key subject for general biology in its present stage of development. It involves questions of genetics, infection, epigenetics, biochemistry, immunology, endocrinology, virology, pathology, clinical medicine, public health and human ecology, and poses in arresting form the problem of their due interrelation. Many authors have pointed out that cancer research will profit from utilizing biological methods and ideas: it is now becoming clear that biological science will profit from the incorporation of the cancer problem in its purview. The hope that my survey may help in securing this amalgamation of cancer research with biology is my justification for the publication of this book.

Acknowledgments

I wish to thank Dr. Rhoads and the Sloan-Kettering Foundation for the invitation which originated this survey, Professor Fox for his encouragement and editorial help in preparing the two articles for *Biological Reviews*, Messrs. Allen and Unwin for their care and efficiency in the publication of this book, and Dr. Peyton Rous for first having made me aware, 45 years ago, that cancer was not a purely medical problem.

The work would never have been accomplished without the helpful co-operation of many of my biological colleagues. I am especially indebted to Professor I. Berenblum, Professor C. D. Darlington, F.R.S., Dr. Honor Fell, F.R.S., Dr. Harriet Ephrussi-Taylor, Dr. E. B. Ford, F.R.S., Professor A. C. Haddow, Dr. Peo Koller, Professor Peter Medawar, F.R.S., Dr. Joseph Needham,

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London, June 1957

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A. Comparative and Genetic

I

INTRODUCTION

1. DEFINITION OF THE SUBJECT

CANCER is not merely a medical problem: it is a biological phenomenon, whose elucidation is bound up with advances in a number of key fields of present-day biology. Like most biological phenomena, it is multi-causal: there is no single 'cause of cancer.'

A general survey of cancer needs to take account of a number of different aspects; (1) descriptive and comparative; (2) physiological and biochemical; (3) genetical; (4) evolutionary; (5) epigenetic or developmental; (6) practical and applied; (7) historical. I shall not attempt to deal with those aspects which (like carcinogenic agents, occupational cancer, hormone-dependent tumours, clinical treatment, biochemistry)[1] have been exhaustively reviewed, or are beyond my competence. Nor shall I attempt to deal with the historical aspects, beyond mentioning the fact that one of the main contributions of research in the past 70 or 80 years has been the disproof of a number of theories as to the nature and causation of cancer. We now know that cancers and other neoplastic growths are not parasitic; not caused by bacterial infection (though in one case, crown-gall in plants, initiated by it); not normally caused by mitotic irregularities; not due (except possibly in a few rare cases) to Cohnheim's 'embryonic rests'; not due to dedifferentiation, in the sense of having reacquired pluripotency; not exclusively a disease of old age, of civilization, or of man alone. Finally, cancer is not a biological entity, like chromosomes or paratyphoid B, or natural groups such as Insecta or Amphibia. It merely denotes a category;

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[1] E.g. Greenstein (1954). Oberling (1952) gives a comprehensive and Hieger (1955) a shorter semi-popular review of the general cancer problem: see also Shimkin (1950).

the term cancer is a convenient label or pigeon-hole for a number of pathological tissue-conditions exhibiting certain common characteristics, but all unique in certain other respects. Indeed, it is not *a* disease at all, but an assemblage of many different diseases which have certain symptoms in common.[1] A century ago medical men spoke of 'fever' as a disease. Now they no longer do so. Something of the sort is happening with cancer during the present century.

Cancer is sometimes used as equivalent only to carcinoma, but this seems an undesirable purism, and I shall employ the term *cancer* to denote all malignant tumours. Cancer in this general sense thus includes both carcinomas and sarcomas, as well as leukaemias, ascites tumours, etc., which can only be rather artificially fitted into the traditional nomenclature. The categories of sarcoma and carcinoma, again, are not in any operative sense entities, though their retention has clearly some value for clinical and descriptive purposes. Various authors, such as Fennell and Castleman (1955), wish to establish a category of non-invasive malignancy—'carcinoma *in situ*' —on purely histological grounds. This seems a regrettable example of specialist scholasticism. Cancer (malignancy) must be defined operatively in terms of what the tumour cells do, not what they look like; otherwise the term ceases to have biological meaning.

But I must now say something of what cancer *is*, rather than what it is *not*. Cancer, in the general usage of malignant tumours, may be defined as the assemblage of tissue growths which share the following characteristics: (1) replication of abnormal tissue type, but frequently with irreversible change towards greater malignancy; (2) non-limited growth by proliferation, within the body as well as in tissue culture; (3) some degree of hypoplastic (anaplastic) dedifferentiation, as shown by loss of normal histological structure and of physiological capacities;[2] (4) partial or often complete lack of organization; (5) invasion and erosion of surrounding tissues, often accompanied by (6) metastasis. The last two properties characterize the malignancy of cancers. The others, which are common also to benign tumours, are basic to the genesis of all pathological neoplastic growths. Thus the cancer problem must be regarded as merely a part of the more general tumour problem or *neoplasia.*

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[1] Willis in his text-book (1953, p. 66) has a section headed, 'Cancer: an ill-defined heterogeneous group of diseases.' McKenzie (1955) says that every individual breast cancer is 'sui generis.' Foulds (1940) states that every tumour is 'specific.' Korngold (1957) has shown that human tumours of the same organ and type may differ in their antigenic properties. See also Berenblum (1956).

[2] Furth (1953, 1955) claims that histological and physiological dedifferentiation is not a necessary accompaniment of tumorigenesis, but may be absent in certain conditioned tumours whose unlimited proliferation is caused by the absence of a normal restraining agency in the host, so long as they remain endocrine-dependent: see pp. 109–110.

Neoplasia is a process. Neoplasia (to anticipate later conclusions) depends on a deviation of metabolism in a particular tissue. This results in a complex process of abnormal development, involving a sequence of interdependent steps or sub-processes. In most cases these fall into two main phases, the pre-autonomous (often called pre-cancerous) and the autonomous. Autonomy is the chief diagnostic character of tumours. The deviated tissue of a tumour has become autonomous in respect of proliferation and of organization; it is no longer subject to the mechanisms normally controlling and limiting proliferation, nor to those which normally integrate tissues into functional organs. The rate of autonomous neoplastic proliferation is almost (but not quite) always higher than that of the parent tissue, and usually increases stepwise by orthoselection during the neoplastic process. Neoplasms behave as parasites, and the neoplastic process often leads to death. Some benign tumours occasionally cause death by their indirect effects; malignant tumours are usually lethal, owing to their capacity for invading and destroying normal tissues and for metastasis.

2. HETEROGENEITY OF TUMOURS

Once the neoplastic process has crossed the threshold of autonomy, the resultant tumour can be logically regarded as a new biological species, with its own specific type of self-replication and with the capacity for further evolution by the incorporation of suitable mutations. From the angle of biological classification, all tumours, whether of plants or animals, could then be regarded as constituting a special organic phylum or major taxonomic group, with the following unique characteristics: (1) universal (and in nature obligatory) parasitism, but with the parasite always (in nature) originating from its host; (2) some loss of supracellular organization; (3) lack of limit to proliferation; (4) (*a*) in most cases each individual tumour is the equivalent of a biological species, so that the number of species is the same as the number of individuals; there is no evolutionary divergence of an original type, and each species becomes extinct on the death of its host; (*b*) in certain virus-induced tumours, and in tumours maintained artificially by grafting, the strain of tissue is the equivalent of the species, and a certain amount of evolutionary divergence may occur in sub-strains. The assemblage cannot be further classified by the usual taxonomic procedure of division according to groups of (presumed) common descent. Classification by taxonomy of the host species, supplemented by specification of the etiology and of the tissue of origin, would be merely a convenient form of detailed pigeon-holing.[1]

To set up a special taxonomic group of this sort in practice would be useless pedantry; but to do so in theory is a useful exercise of the scientific imagination. In any case, we must expect a truly enormous degree of heterogeneity among tumours. (*a*) *Genetic heterogeneity* (see p. 36 f.). (a_1) *Taxonomic heterogeneity.* This will reflect the following facts: The constitutional (genetic) differences between different taxonomic groups of host—specific, generic and of higher order up to differences in phyla and kingdoms. The constitutional peculiarities of higher plants will be reflected in peculiarities of plant tumours; insectan tumours will show certain general differences from vertebrate tumours. (a_2) *Intraspecific genetic heterogeneity.* (i) Within host species: this involves differences, quantitative and qualitative, in the proneness (susceptibility) of different strains and individuals to cancers of different types, and different reactivity to viruses and other carcinogenic agents. (ii) Within tumour species: this involves extensive variance in the cell-population of a given tumour strain (p. 44). (*b*) *Epigenetic heterogeneity*, depending on the irreversible differentiations of different tissues, organs and regions within the host organism. The somatic constitutional peculiarities of epithelia as against other tissues will be reflected in the general difference between carcinomata and sarcomata; those between, say, thyroid and mammary gland tissue in differences between the types of tumour to which they give rise. (*c*) *Environmental heterogeneity.* Some tumorigenic processes appear to be highly modifiable by conditions of the external and internal environment. Thus certain virus tumours in plants are not produced except after traumatic injury; the incidence of some *Drosophila* tumours is alterable by temperature, diet, oxygen-content, etc., while in endocrine-dependent tumours, upsets of the endocrine balance may have striking effects; the incidence of different types and sites of tumour in man as a species is very different from that in any other mammal, apparently because of his different dietary and other habits; and it varies markedly within the human species in relation to occupation, habits and environment.

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[1] See Greenstein (1954, ch. 2) on the general phenomena and taxonomy of cancer.

II

COMPARATIVE DISTRIBUTION OF NEOPLASMS

A FIRST step in the analysis of any biological problem is descriptive and comparative study; a natural history of tumours is a necessary basis for cancer research. Neoplastic tumours are widespread in the organic realm. In higher plants we have crown-galls, wound tumours and genetic tumours, and experimental tumours in higher fungi. Benign and malignant tumours are known to occur spontaneously in all five classes of Vertebrata, in Crustacea, Arachnida and the insects, probably in Tunicata, cephalopod and lamellibranch Mollusca and annelid worms. They have been experimentally induced in mammals, birds and amphibians among vertebrates, in Coleoptera, Diptera and Orthoptera among insects, and in Crustacea. Genetically determined tumours are known in wide crosses in fish, moths and higher plants, and in selected breeds or inbred lines in mammals and in *Drosophila.*

1. VERTEBRATES

For tumours in mammals and birds we have older studies such as that of Bland-Sutton (1890), and the more recent general survey of Willis (1953, ch. 6), together with Feldman's book (1932) and Cotchin's papers (1952, 1954, 1956, 1957) on neoplasms in domestic animals, and Fox's (1923) chapter on neoplasms in captive wild species.

Among the interesting facts described by Bland-Sutton I may mention the large hyperplastic bone-growths caused by mechanical injury to deer antlers in velvet; the enormous cutaneous horns occasionally arising from warts in mammals, including man, and birds; the feather tuft normally arising from the meningocoelic craniums of birds; the abnormal localized growth of hair in cases of spina bifida in man (his fig. 12); the frequency of bone-tumours in the fish *Chaetodon* (his fig. 105); the tumour-like growths produced by the fungal infection actinomycosis in cattle; and a remarkable tumour in the pouch of a phalanger. Throughout he stresses the frequent analogy of abnormal hyperplasias and tumours with the normal products of excessively high growth-rate such as deer antlers or the horns of rhinoceros and Bovidae. Such areas are often sharply delimited from surrounding areas where similar tissues have a low proliferation rate; yet the growth-pattern and resultant form of the organ is determined by an invisible growth-gradient (growth-field) in the entire region, as in rhinoceros horns (Huxley, 1932, pp. 150–2). In young bull calves, when the lateral high-growth areas (horn-cores) are transplanted to the mid-line of the head's growth-field, they fuse and form a single straight horn (Dove, 1935, 1936). Analysis of such phenomena, notably of the basis for sharp local differences in proliferation-rate, and the application of carcinogenic agents to the horn-cores of young ruminants and to deer antlers in velvet, should be relevant to the cancer problem.

Dr. Cotchin has drawn my attention to the horn-cancer which affects domestic cattle and buffaloes, but only in India and S.E. Asia, where it constitutes 23% of all bovine tumours (Nair and Sastry, 1954: see also Cotchin, 1956; Kulkarni, 1953; Burggraaf, 1935). Most cases are squamous-cell carcinomas, which may invade the frontal sinus and metastasize. Its frequency in bullocks is six times that in cows, which are much less often employed for traction or ploughing; that yoke-pressure may be a major initiating cause is suggested by the absence of the disease elsewhere, though ocular cancer is present (Russell, Wynne and Loquvam, 1956).

Willis (1953) states that, as one would expect, tumour incidence in vertebrates increases not so much with time as with age, ageing taking place at different rates in different forms. Thus most mice with spontaneous tumours are from 7½ to 20 months old,[1] most rats from

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[1] In the same strain of mice different tumours develop at different ages; e.g. in breeding females, mammary tumours at 11·8 months, lung tumours at 7·5 months (Hieger, 1955).

2 to 3 years, while the great majority of dogs with tumours are over 6, and of horses over 10 years old. Dr. Rhoads (*in litt.*) informs me that the same chemical carcinogen produced cancers in a few months in mice, but one to several years in monkeys. It would be of great interest to compare the age-incidence of tumours in birds and mammals of comparable size, to see whether longevity or rate of maturation is the more important factor; the birds live much longer but reach maturity and adult size in about the same time.

In whales tumours appear to be rare (Rewell and Willis, 1949, 1950). Granulosa-cell ovarian tumours were the commonest (three out of seven); this may be correlated with the very large size of cetacean corpora lutea. The tumours were small relative to total bulk; for instance, two benign tumours of 8 and 4 cm. diameter in the (enormous!) tongue and the uterus respectively. This is apparently not merely due to the difficulty of vascularization with increasing size, since much larger tumours, benign and malignant, commonly occur in man and other large land mammals.

Willis concludes that, though all warm-blooded vertebrates are 'probably susceptible to tumours . . . it is clear that species do differ intrinsically in their proneness to tumours in general, and to tumours of specific kinds.' Thus horses are more, sheep and pigs less, tumour-prone than cattle;[1] and dogs more so than cats. Tumour incidence and inducibility is high in rats and mice, but low in rabbits and primates, and very low in guinea-pigs (*Cavia*). In both these latter types the experimental induction of tumours is also difficult. Thus less than 0·01 mg benzpyrene can induce tumours in mice, but monkeys are refractory to 15 mg benzpyrene pellets (Hieger, 1955, p. 42). However, N. Petrov (cited in Anon. 1956*c*; and see Petrov, 1956) states that he has produced 'osteogenic sarcoma' in 1 out of 25 rhesus monkeys after 3 years by injecting methylcholanthrene into the bone-marrow, and in 3 out of 7 monkeys after 10 years after intra-medullary implantation of radium. Suguira, Smith and Sunderland (1956) have produced papillomas followed by malignancy in 3 out of 6 rhesus monkeys treated with repeated applications of a new petroleum derivative, a high-boiling catalytically-cracked oil.

This substance also produced tumours in mice and rabbits, but not in rats or guinea-pigs (to the latter of which it was highly toxic). The times to 1st papilloma for mice, rabbits and monkeys were 29, 26, and 322 days, and to 1st cancer 70 days, 2 years and 3¾ years. Thus rabbits, though larger than mice, developed papillomas more rapidly (after 42 days two-thirds of them had papillomas, while only half the mice had papillomas after 70 days). In spite of this, the mice developed cancers much more rapidly—a fact that may be partly due to their receiving a larger dose per gram body-weight. In rabbits, skin cancer can be induced by a carcinogen to which mice are refractory (Hieger, 1955, p. 43). Among birds, budgerigars (*Melopsittacus undulatus*) have a high incidence, especially of renal tumours, a fact confirmed by Fox (1923), and of tumours of the pituitary (Schlumberger, 1954).

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[1] Feldman (1932) comes to the opposite conclusion. Probably the discrepancy is due to Willis including the melanomas of grey horses (p. 51).

In birds, according to Willis (1953, p. 267), almost all neoplasms, spontaneous or induced, are sarcomata, and carcinomata are very rare. However, Campbell (1945) found that in 2,063 fowls examined in a routine diagnosis laboratory there were 386 cases of neoplasia, 77 of which were carcinomata. He considers (*in litt.*) that the apparent rarity of carcinomata in fowls is due to the fact that most of the birds are not allowed to survive to the age at which carcinoma is likely to appear (i.e. from the third year upwards). However, this would not apply to birds of other species kept in zoos and aviaries.

Cohen and 'Espinasse (1957) after mentioning a previous case of carcinoma in a Pennant's parakeet, report a basal-cell carcinoma, apparently derived from basal cells of a feather-germ, found in a 9-year-old Brown Leghorn capon. The bird had been given four intradermal injections of oestrone, and a dose of 500r X-radiation.

Cotchin (1952) states that 'it is generally agreed that the order of frequency with which tumours occur (in domestic animals) is probably: dog—common; horse and ox—much less common [presumably excluding the melanomas of grey horses]; sheep and pig—rare; with the cat falling somewhat behind the dog.' However, the precise incidences are difficult to estimate from existing data, and Cotchin rightly asks for organized statistical inquiry on the subject.

In dogs, as in other species (Cotchin, 1954), tumour incidence in animals brought for examination rises sharply with age, from 1·5% in those under 1 year to 10% between 6 and 10 years. The site of cancer in dogs differs markedly from that in man, uterus, alimentary tract and lungs being all rare sites in dogs and common in man, while cancer of the canine tonsil is common. Dogs differ also from cats (Cotchin, 1952, 1956, 1957), where carcinoma of the tongue and oesophagus is common (as in man), but there are fewer types of skin tumour, and tumours of the thyroid and of the male reproductive system are rare. This last fact may perhaps be related to the frequency of castration in male kittens. Fox states that skin and mammary cancers are much less common in rats than in mice. Further details may be found in Feldman (1932), including the estimation of tumour incidence (apart from leukosis) in the domestic fowl at 2–3% in birds from 6 to 18 months old, and 6–10% in old birds. When leukosis is included, fowls are the most cancer-prone of all animals, 20 or even 40% of some flocks dying of it (Hieger, 1955, p. 16). The combs of fowls, which show marked endocrine-dependent allometry, should provide interesting material for experimental tumorigenesis.

In animals in the Philadelphia Zoo, Fox (1923) has recorded neoplasia in seven orders of placental mammals, including elephants, edentates and Old World and New World monkeys and lemurs among primates; among marsupials he records neoplasia in Dasyuridae and Peramelidae (but not Macropodidae), and in New World opossums. In birds he has recorded neoplasia in nine orders or supra-ordinal groups—ratites, owls, hawks, gamebirds, doves, rails, ducks, parrots and passerines. It appears to be especially common in the true parrots or Psittacidae (twenty-three cases as against twenty-one for all other birds), notably, as already mentioned, in budgerigars. As this bird is easily reared and bred, and we have considerable knowledge of its genetics, it should prove a valuable experimental species for cancer research. The uterus was the commonest site in mammals (though rather less so in carnivores), the kidney in birds. The total tumour incidence in 5365 autopsied captive mammals was 1·7%, in autopsied birds 1·2% (much lower if budgerigars are excluded).

Intergeneric and even inter-class tumour grafts will 'take' readily in the large cheek-pouches of the golden hamster (*Cricetus auratus*) (Patt, Handler and Lutz, 1952). It is interesting to recall that all golden hamsters known to man are descended from a single pregnant female, so that it is possible that a peculiar genetic constitution is involved. It would be interesting to see whether other species of rodent with large cheek-pouches provide equally favourable sites for interspecific grafting.

Too little is known about the differences in general and specific tumour-incidence in different breeds of domestic animals. Cotchin (1954) states that among dogs, retrievers have an unexpectedly high and pekinese and chows an unexpectedly low general tumour-incidence, that Scotch terriers are especially prone to melanomas, Boston terriers to mast-cell sarcomas, and all breeds with a bull strain to brain tumours; and mentions the suggestive fact that sarcomas of limb-bones are relatively frequent in large breeds of dog.

Schlumberger and Lucké (1948) give comprehensive data on neoplasia in poikilothermic vertebrates. In reptiles recorded tumours are few. A fair number are noted in various forms of the notoriously long-lived Chelonia; only two in Crocodilia; and only six in Lacertilia. In one of this latter group attempts at tissue-culture and transplantation to the eye-chamber of the same and other species were unsuccessful. In Chelonia tumour-like growths are sometimes associated with trematodes (fibromas) or with leeches (papillomas). In the latter case, the leeches' hirudin may have directly or indirectly stimulated the tumour's growth. Nine tumours occurred in snakes, several being malignant melanomas; attempts at chorio-allantoic grafting of one of these failed, apparently because the python tissue was toxic to the chick embryos. Melanomas have been experimentally induced in reptiles: this should be repeated in some easily kept species of tortoise, lizard and snake.

In Amphibia only a few spontaneous tumours are reported from urodeles, which is surprising in view of their permanent retention of regenerative capacity. Three were in the long-lived giant salamander (*Megalobatrachus maximus*), and two, both melanomas, in axolotls; one aquarium strain of this species showed a 'hereditary tendency to melanoma.' A skin carcinoma in *Triton alpestris* appears to be transmissible by viroid particles as well as by inoculation of fragments; a high proportion of animals of this (but not of other) *Triton* species developed the tumour on being placed in a 'contaminated' tank.

In Anura twenty-two cases are reported, twenty-one in *Rana*, the twenty-second in the horned frog (*Ceratophrys*). The most interesting is the metastasizing Lucké renal carcinoma of the leopard frog (*Rana pipiens*), discussed on p. 77.

Tumours have been chemically induced in adult newts of the genera *Triton* (Koch, Schreiber and Schreiber, 1939) and *Triturus* (Breedis, 1952), and in larval frogs, *Rana pipiens* (Briggs, 1940, 1942). In *Triturus* tar injected into the forelimb often gave rise to supernumerary limbs or parts of limbs. Methylcholanthrene produced one unorganized sarcomatous tumour, and one poor accessory limb which later developed a sarcoma at its base; both were readily transmissible by grafting.

In the pre-gastrular stages of Anura tumour-like growths have been induced by late fertilization (p. 99; Witschi, 1952) and by immersing the eggs in weak zinc acetate solutions (Lallier, 1955). Grafting of entire early embryos or fragments of embryos into later stages gives rise to disorganized tumorous growths (p. 99).

The advanced state of our analytic knowledge concerning embryology, larval development, metamorphosis and regeneration of Amphibia makes them extremely suitable material for cancer research.

In teleost fish, records of spontaneous tumours are much more abundant. This is partly because they are the most numerous group of vertebrates, partly because they are taken in such large numbers for food. Tumours have been recorded in no less than forty-eight teleost families. Among elasmobranchs, recorded tumours are rarer, presumably because these fish are less numerous in species, and more rarely taken for food; but they occur in three families of sharks and dogfish, and in rays (though so far only in the genus *Raia*, where five of the seven records are of skin melanomas).

The following fish should make good material for cancer research. Domesticated goldfish (*Carassius auratus*) exhibit benign and malignant tumours of many types (though, curiously enough, none of the thyroid or of the pigmentary system). The small aquarium fishes of the families Poeciliidae and Cyprinodontidae develop adenomas, thyroid tumours, fibromas, fibrosarcomas, lymphosarcomas, and especially pigmentary tumours (p. 39 f.). With the exception of haemangiomas, tumours appear to be rare in sticklebacks (*Gasterosteus*), but the ease with which these can be kept and bred, and the difference in haemangioma incidence between American and European species (Nigrelli, 1954) is a recommendation for further study. The electric eel (*Gymnotus*) is not exempt from tumorous growths, as is sometimes supposed, but may exhibit skin papillomas.

Some organs of fish not present in land vertebrates may be affected; for instance, the highly vascular parabranchial bodies and the rectal gland (both of unknown function). The commonest benign tumours of teleosts are skin papillomas. The multiple papillomas of the climbing perch (*Anabas scandens*) appear to be transmissible by infection and in many ways resemble mammalian infectious warts. Flatfish seem relatively more prone to sarcoma (*sensu lato*) than other groups. Odontomas of placoid scales are known in elasmobranchs, and of true teeth in teleosts.

Thyroid 'tumours' have been described in a number of teleosts, notably in Salmonidae, but also, for example, in swordtails (*Xiphophorus*) (Aronowitz, Edgar and Gordon, 1952). Many of these growths regress when iodine is added, and seem to be merely hyperplasias (goitres), apparently induced by conditions unfavourable to oxidative metabolism, notably low oxygen-content of the water, to which salmonids are especially sensitive. Others may perhaps be classed as benign tumours, though they simulate malignancy by invading neighbouring tissues such as bone. This is apparently because the thyroid of fish is not encapsulated; benign tumours of the mammalian thyroid may reach an enormous size, but are restrained from invasion by the tough capsule. However, Schlumberger and Lucké conclude that a few thyroid tumours in fish are almost certainly malignant; in one case at least metastasis had occurred. The only other teleosts in which goitres or thyroid tumours are common are the shark-suckers (*Echineis*); the reason for this is unknown.

Nigrelli (1954) has made a detailed study of tumours in North American fresh-water fish. He pays considerable attention to the tumour-like growths caused by parasites, including nematodes, trematodes, cestodes, copepods and sporozoans (Myxosporidia). Some of these growths are obviously mere hyperplasias, but others, especially those induced by Myxosporidia, may be very massive and apparently neoplastic, sometimes even causing metaplasia; these should repay further study. The transmissible viral lymphocytosis of many fish, in which the infected cells hypertrophy to a 'gigantic size' and there may be large proliferation of connective tissue elements, may also have some relevance to the cancer problem.

Nigrelli (1938) has also reviewed the incidence of tumours in aquarium fishes. One of the conclusions that emerges is the need for experimental analysis to determine the share of heredity, environment, and infection in the causation of their tumours.

2. INVERTEBRATES

Tumours in invertebrates present an important and little-worked field of study, as emerges from the review of the subject by Scharrer and Lochhead (1950). As late as 1930 it was asserted by some workers that invertebrates did not, or even for various alleged reasons could not, develop tumours; and even to-day it is sometimes stated that invertebrate tumours are not 'really' neoplasms, or are irrelevant to the study of vertebrate cancer (for *Drosophila* tumours, see p. 47 f.). Such statements appear quite ill-founded. While it is difficult to be sure whether many growths found in nature are true neoplasms or merely hyperplasias, this is largely due to the lack of sufficient expert attention to the problem. More intensive work has shown that various invertebrate (and plant) tumours manifest non-limited growth and lack of organization, and some are malignant, while others have been experimentally induced. It is true that invertebrate tumours often differ markedly from vertebrate neoplasms, and are difficult to fit into the categories of the human 'oncologist.' But this should not surprise us, any more than does the fact that an insect or an echinoderm is built on a different ground-plan from a vertebrate. Indeed, it should be a stimulus to research aimed at eliciting the common factors of all tumorous growths, and at discovering the different ways in which the neoplastic process has broken through the normal barriers to non-limited growth and organizational autonomy in different types of organism.

Among the sporadic reports of spontaneous tumours cited by Scharrer and Lochhead the following may be mentioned. In arthropods a lethal tumour in the grinding stomach of a lobster; large tumours on the thorax of the arachnoid harvest-man *Phalangium opilio*, and of the beetle *Phytodecta variabilis*; a brain tumour in an ant, *Formica pratensis*; a pharyngeal gland tumour in a species of bumble-bee (*Bombus*). The cysts in the hindgut of old winter worker-bees, formed by fusion of giant cells, may perhaps be neoplastic, but certainly demand further investigation. The many genetically determined tumours found in larvae of *Drosophila* and the moth *Pygaera nigra* will be considered in the next section. In molluscs, several large tumours have been noted in the fresh-water mussel *Anodon cygnea;* a very large pericardial tumour in an oyster *Ostrea virginica*; a stalked tumour in a 4-year-old slug *Limax flavus*; and a tumour of the vascular system is reported in the aberrant worm *Sipunculus*.

Tumour-like growths induced by parasites are not infrequent. One of the most interesting is that induced by the gregarine *Monocystis* in the ascidian *Ciona*, which apparently may become malignant. Non-malignant growths associated with a polyhedral virus occur in the spruce sawfly *Gilpinia hercyniae*, and with a sporozoan parasite in the sabellid polychaete worm *Potamilla torelli.*

Finally, tumours have been experimentally induced in several invertebrate groups. In the hermit-crab *Pagurus longicarpus*, Hammett and Hammett (1933) found aberrant unorganized tissue growths arising from mechanical lesions at the amputation site or between the segments of a regenerating right (large) chela, both with and without treatment with *p*-thiocresol, whose sulphhydryl group Hammett considered as growth-promoting. In treated animals the growths were larger and more frequent (100% as against 75%) and sometimes developed at the tip of the regenerating chela in the absence of any injury. Crustacea should provide very rewarding material for work on various subjects relevant to the cancer problem; for example, on regions of high growth-potential, and the growth-gradients associated with them; on compensatory hypertrophy; and the effects of carcinogens, radiation and repeated regeneration on regenerating organs of high and low growth-potential (see Huxley, 1932; Weiss, 1955). The simple or complex growth-gradients in the mantle-edge of molluscs which determine the form of their shells (Huxley, 1932, p. 154 f.) could also be usefully studied.

Needham (1942, p. 260) records how in 1927 he noted the frequency of tumours in the marine polychaete worm *Nereis* in habitats of low salt-content. Thomas later (1930) showed that these were due to degenerated oocytes, which then stimulated connective tissue growth, sometimes of an invasive nature. Inoculation of such worms with *Agrobacterium tumefaciens* (which initiates tumours in plants) caused rapidly growing, invasive and fatal tumours; worms in which no degeneration of oocytes had occurred did not respond. If this is confirmed, the initiation of the neoplastic process is here due to a degenerative change in the animal's own tissues, and *A. tumefaciens* merely extends and exaggerates it, whereas in plants the initiation is due to the bacterium (p. 29 f.). Similar inoculations of larvae of the wax-moth *Galleria* by other workers produced no effects. It would clearly be of interest to repeat and extend this work.

In cuttlefish (*Sepia*) subcutaneous implantation of 1-, 2-, 5-, 6-dibenzanthracene is stated to have produced a large tumour after a few days (Jaquemain, Jullien and Noel, 1947); this short lapse of time is highly improbable, and the work needs confirmation.

What appear to be hormone-dependent tumours have been produced in stick-insects (*Dixippus*) by ablation of the corpora allata, the endocrine glands whose secretion of 'juvenile hormone,' responsible for preventing the development of an insect into a more highly differentiated or more adult phase, decreases in amount during larval or nymphal life. The effects of ablation could be corrected by reimplantation (Pflugfelder, 1939, 1948). Tumorous growths of a rather different nature are induced in auto-specific embryonic tissue grafted into the head region of animals whose endocrine balance had been upset by ablation. Early ablation sometimes leads to various types of tissue degeneration, in one of which degenerated mesenchyme cells may attack the nerve-cord and cause death. At other times it leads to proliferation and tumour-formation, again of various types and of various degrees of severity, some apparently malignant. Both effects may occur in the same individual. Limb regeneration occurring after ablation is incomplete, and is accompanied by tissue degeneration and tumorous proliferation. Differentiation may also be affected; ablation followed by excision of the compound eyes leads to the differentiation of rudiments of simple eyes (ocelli). This species clearly offers great advantages for further work.

L'Hélias (1957) finds (in other phasmids) that proliferation is controlled by 2 pteridinic compounds, one (related to folic acid), accelerating it, the other (secreted by the corpora allata) retarding it. In the absence of the retarding factor, since differentiation is inhibited and proliferation increased, injection of folic acid or the accelerating factor produces unco-ordinated proliferation and sometimes tumours. Pierid pupae thus injected during diapause (when the corpora allata are inactivated) produce melanomas resembling the spontaneous tumours of *Drosophila* at the site of injection. These later spread and may form metastases.

Unique results have been obtained by Scharrer (1945, 1949) by cutting the recurrent nerve in the large orthopteran insect *Leucophaea*. In about 75% of cases this operation induces tumours in the organs innervated by the nerve—the salivary glands and reservoir and the anterior portion of the gut. The tumours may grow to a large size and not infrequently cause death; sometimes they become malignant, invading neighbouring tissues and even perforating the body-wall. Susceptibility to such tumours is higher in females than in males or castrated females; this may be correlated with the existence, in the suboesophageal ganglion of females, of special neuro-secretory cells which become distinguishable only after ovariectomy, and presumably stand in some endocrine relationship with the female gonad (Scharrer, 1955). In guinea-pigs damage to the sympathetic nervous system may produce tumours (p. 100). Such work may shed important light on trophic nerve-action.

3. PLANTS

The subject of tumours in plants has been reviewed by White (1951), by Riker and Hildebrandt (1953), and by Braun (1952).

The best-known plant tumours are the crown-galls, which are initiated by infection with *Agrobacterium tumefaciens*, and occur naturally in a great range of dicotyledonous species with not-too-acid cell-sap.

Though *A. tumefaciens* infection is necessary to initiate a crown-gall tumour, this can then produce secondary growths in other parts of the plant. These (and also the inner portions of primary galls in certain groups of plants) are sterile (bacterium-free) and are serially transplantable, both in the parent and related species of plants, and can be cultivated *in vitro*; thus the crown-gall tumour, once initiated, is a self-reproducing tissue-species.

Standardized tissue masses (disks of cambial-adjacent phloem from carrot roots) if infected with *A. tumefaciens*, will produce tumours (Klein, 1955). Some strains of carrot give a strong tumour-forming reaction, others a weak one. Sterile wound-juice from 'strong' strains had no effect on the reaction of 'strong' disks, but marked effect on 'weak' ones, shortening the time to the appearance of tumours and increasing their final weight; thus genetic differences in tumour-proneness exist.

Crown-gall formation clearly involves an alterative phase followed by one of non-limited growth, in each of which a distinct process is at work. The alterative phase depends on infection with *A. tumefaciens*. It is further subdivisible into two sub-periods (Klein, 1953). The first or 'conditioning' sub-period, lasting less than 24 hr., leads to the production of a specific tumour-inducing principle or substance (T.I.P.) by the bacteria; in the second, this induces a high production of heteroauxin by the plant cells of the infected region, which leads to their high growth-rate and to their liberation from close mechanical contact with their neighbours (White, 1951; and see p. 105). This in turn leads to the second main phase, of visible autonomous tumour growth, with the further possibility of forming secondaries.

Some further points may be noted. The tropical *Vinca rosea* will survive for several days at a temperature of 45° C., which is sufficient to kill *Agrobacterium tumefaciens*. If a plant of this species of *Vinca* is infected and then exposed to 45° C., it is cured of its infection, but will still produce crown-galls, showing the autonomy of the tumour once initiated. The bacterial T.I.P. is also temperature-sensitive from the second to the fifth day after inoculation, being destroyed as fast as formed at 32° C. and above (Klein, 1953). In certain plant species, tumour formation will only occur at or below 25° C.: a minimum time of 10 hr. at this temperature is required for the T.I.P. to exert its effect.

Braun (1952) cites work showing that tumorous buds may lose their tumorous character and revert to a normal growth-rate after two graft-passages on normal plants. This suggests that a plasmagene may be involved, which, like Kappa in Paramecium (p. 88) cannot keep pace with excessively rapid growth, and is thus "bred out" of the system.

Attenuated strains of *Agrobacterium tumefaciens* can be obtained. These will not produce primary tumours at the site of inoculation, nor certain abnormal types of organ-growth often associated with crown-gall, but do induce 'secondary' growths on the leaves, suggesting that the level of auxin-production of the host tissues may play a part in tumorigenesis. This was experimentally confirmed on tomatoes by using a combination of (1) inoculation of attenuated bacteria, and (2) smearing with a paste containing indole-acetic or naphthalene-acetic acid; this combination induced tumours, though neither treatment alone was effective.

Of considerable interest are the relations between *in vitro* cultures of normal and tumour tissue on the one hand and various kinds or concentrations of auxins on the other. In general, normal tissues respond to auxins by rapid growth at low concentrations, by root formation at medium concentrations, and by hyperhydric swelling and death at high ones. Tumour tissues give no growth response to auxins, but may give the rooting response at much lower concentrations than normal tissues. Tumour tissues, both of crown-galls and of *Nicotiana* (p. 38), will grow actively in media without auxins. When pieces of normal and tumour tissue are made to fuse in a simple nutrient medium incapable of providing for continual growth in normal tissues, the tumour tissue 'induces' its own type of growth-rate and histological pattern in the normal tissue. The tissues of different species differ in their auxin and vitamin requirements. Rose tissues are unusual in requiring no additional heteroauxin for growth. In crown-galls of sugar-beets (*Beta*), Winge (1927) found frequent changes of ploidy, either increased (up to octoploid) or decreased (from tetraploid to diploid).

Significantly, some plant tissues (e.g. carrot, Virginia creeper), when cultivated for a long time on a medium containing an auxin (e.g. indole-acetic acid), acquire some growth-properties of tumour tissue; they are able to grow in media without auxin and become capable of inducing growth in normal tissue-masses fused with them (see above), though not at quite the rate induced by crown-gall tissue. Sometimes the change is localized and sudden, as if due to some kind of mutation. According to Riker and Hildebrandt, though *Agrobacterium tumefaciens* itself forms auxins or their precursors, its T.I.P. appears to be, or to be associated with, a large molecule with a protein-like structure. See also Gautheret's interesting book (1949).

Crown-gall plants produce 'embryonic tissues' as well as tumours. Fragments of these in culture grow faster than normal but slower than tumour tissue. When grafted to normal plants, they produce 'embryonic overgrowths'; but repeated grafting leads to a reversion of the tissue to normality, possibly through the dilution of the growth-factor by continued rapid growth. Various substances, such as folic acid analogues and 8-azoguanine, markedly inhibit growth in tumour tissue and in normal embryos and roots *in vitro.* Crown-gall can sometimes be 'cured' by killing the bacteria with penicillin. Levine (1951*a*) found that the *in vitro* growth of roots was somewhat stimulated by various chemical carcinogens.

Few experiments seem to have been made with radiation. However, Gunckel and Sparrow (1954), with continuous ionizing radiation, obtained various abnormal growth-responses, including the production of roots by stem tissues, and of many tumours. They consider that there is normally a balanced system of growth-promoting and growth-regulating or growth-inhibiting substances, and that radiation damages or destroys the latter.

Link, Wilcox, Eggers and Klein (1953) studied the growth-balance between normal and tumour tissue at various levels of nitrate-nitrogen nutrition. In general, the growth of tumours is only slightly affected by the nutritional state of the host; suppression of tomato development by maleic hydroside has no effect on the growth-rate or final size of tumours. However, on low nitrogen nutrition, the depression of the host's metabolism permits a considerable size increase of the competing tumours, which in some cases may become 'dominant,' persisting even after death of the host apex.

Beardsley (1955) finds that crown-gall tumours are induced by cell-free preparations of *Agrobacterium,* perhaps by a liberated phage. Klein (1953) claims that the agent is 'not a true virus,' and others state that only temporary growths are thus inducible. Klein and Klein (1953) have shown that metabolic products, and in some cases purified desoxyribonucleic acid, from virulent *A. tumefaciens* can transform related bacteria; they irreversibly confer virulence (i.e. the capacity to initiate crown-gall) not only on avirulent strains of the same species, but on normally avirulent bacteria such as *Radiobacter* and *Rhizobium.*

The general conclusion seems to be that *Agrobacterium tumefaciens* produces some chemical carcinogen which induces an irreversible deviation of metabolism in the adjacent plant cells. The deviated tissue produces an increased amount of auxin, and so acquires a high growth-rate and also becomes autonomous. The general parallel with the cancer process in animals is striking, though the chemical details are clearly quite different: the compounds which are carcinogenic for mammals appear to have little effect on plants (Levine, 1951*b*). The tumorigenic effect of excess auxin is closely paralleled by that of excess hormones in animals (pp. 109, 110).

White and Millington (1954) and Reinert and White (1956) have described an interesting tumour of white spruce, *Picea glauca*, for whose development three consecutive factors appear to be necessary: (1) an insect lesion, (2) a 'sensitizing' agent such as *Agrobacterium* and (3) exposure to salt spray.

Thomas, Evans and Hughes (1956) have induced striking tumours, cytologically similar to animal tumours, in basidiomycete fungi (*Collybia* and cultivated mushrooms, *Psalliota*) by diesel-oil vapour and exhaust fumes and by tar fumes. Diesel-oil vapour induces tumours in over 80% of treated mushrooms within 10 days. The tumour tissue *in vitro* usually shows an increased growth-rate and some at least of the metabolic peculiarities of mammalian tumours. This opens up a promising new field for research and should provide abundant tumour tissue for physiological and biochemical studies.

True virus cancers do exist in plants (e.g. sweet clover and sorrel, *Rumex acetosa*). These are striking woody tumours, induced in the first instance by wounding. Neither virus nor wounding alone is effective, but when a group of cells which has been invaded by the virus is subjected to a traumatic stimulus, it is incited to rapid and disorganized tumorous growth (White, 1951). Genetic proneness to virus tumours varies considerably. Growth of virus tumours *in vitro* is enhanced by alkaline hydrolysed ribonucleic acid but decreased by desoxyribonucleic acid, and markedly inhibited by alanine and related substances and by the triazolo analogue of guanine (Nickell, Greenfield and Burkholder, 1950). Unlike crown-gall, virus tumours are also inhibited by the growth-preventing 'anti-auxin,' maleic hydrozide (Nickell, 1953). Here, as in animals, the neoplastic process can be initiated by several quite different agencies. Other viruses can produce abnormal hyperplasias—for example, the enations due to some strains of tobacco mosaic virus.

Allsopp (1957) obtained normal development of two species of liverwort from spores on an agar medium containing only inorganic nutrients: when glucose was added, a minority of cultures developed into irregular undifferentiated tissue-masses which continued to grow at a high rate for over 6 months. (The addition of glucose had no effect on similar cultures of Marsilea and various Bryales.) In *Reboulia* the tissue is all-green and consists of small clumps of isodiametric cells which occasionally form cylindrical outgrowths resembling protonema. In *Fossombronia*, there are patches of colourless as well as of green tissue. In both cases, normal differentiation and growth-rate sets in on transference to a non-glucose medium. It would be interesting to try the effect of excess auxins and of infection with *Agrobacterium*.

Attention must now be drawn to the non-autonomous growths of plants, such as galls and root-nodules. Allen, Allen and Newman (1955) found that many benzoic acid derivatives can cause galls of various sorts. One induced in the rootlets of kidney beans 'pseudo-nodules' very similar to the nodules normally caused by symbiotic nitrogen-fixing bacteria, while Needham (1942) records that the nitrogen-fixing bacteria produce an auxin. White (1951) records numerous substances capable of inducing intumescent galls on various plants.

The study of plant galls should throw light on the distinctness of the factors causing escape from limitations of proliferation from those leading to escape from organization, since some galls are merely irregular disorganized growths, while others, though entirely abnormal, show a sharply limited growth together with a specific organization.[1] Furthermore, there is a high degree of specificity in the type of gall induced by different gall insects, several distinct types often occurring on one species of plant. When one species of insect attacks several species of plant, the type of gall is usually constant, though with minor differences according to host. For details, reference should be made to Küster (1911) and Ross and Hedicke

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[1] I recall being much struck in Texas by the galls of the Sweet Gum (*Liquidambar*), which are hollow spheres, their walls joined by radial struts to a central chamber for the insect larva.

(1927). We know that no insect galls so far tested are truly tumorous, in the sense of having acquired the capacity for non-limited growth. When grown *in vitro* both gall and normal tissues rapidly develop cork, show liquefaction, and then cease growth.

Long ago, Goebel in his great work *Organographie der Pflanzen* stated that the study of gall-formation was of crucial importance for developmental morphology. In fact, fundamental advance in this field has been disappointingly slow; though Needham (1942) in his useful summary records that in some groups the agent is supplied by the developing larvae, but in others by the ovipositing adult. In some cases (Homoptera) the agent appears to be derived from the salivary glands, in cynipid larvae from the Malpighian tubules; while sawfly eggs killed with a hot needle directly after laying are still capable of inducing typical galls. Early attempts to isolate the specific gall-inducing substance from the parent or larval insects have failed. Recently, however, Hovanitz (1956) has successfully caused pre-gall formation (abnormal proliferation and growth of callus tissue) by inserting preparations from larval and adult gall-insects. Preparations from an insect which normally produces only leaf-galls may induce gall-like swellings in stems, as well as in leaves. It is encouraging that the problem is being attacked afresh with the aid of modern methods and in the light of recent discoveries in biochemistry and epigenetics.

III

TUMOUR GENETICS

1. INTRODUCTORY

I NOW come to the genetics of tumours. Although the implications of this subject are of outstanding importance, it is still bedevilled by the ghosts of dead ideas about heredity, which continue to haunt the popular and I fear to some extent the medical mind.

The idea of biological inheritance was in the first instance taken over from the facts of social inheritance, of title, land or wealth; it was tacitly assumed to mean the transmission of recognizable characters from parent to offspring. However, neo-Mendelian research has made it clear not only that characters as such are never transmitted, but that their manifestation depends on the interaction of a large number of genes with each other and with the external and internal environment. With the emergence of the concept of the gene-complex, 'heredity' as a subject for biological research was decently interred, and the science of genetics was born. As Darlington (1954) says, 'heredity' in the popular view denotes the resemblance between parent and offspring in a cross-breeding population. Experiment has revealed that this is merely one manifestation of *genetic determination*. Family pedigrees can still be of great value, and are indeed indispensable where, as in man, pure strains are unavailable and experiment impossible; but they are of value only when interpreted in the light of concepts and principles derived from the experimental breeding of large numbers of organisms characterized by known genetic differences.

2. SPECIFIC AND RACIAL DIFFERENCES

In regard to cancer we find marked differences of type and incidence of tumours in related species or subspecies; and these must have a genetic basis. Thus, guinea-pigs are much less tumour-prone than mice (p. 20) and ducks much less than fowls. Another fact which must be genetically determined is the relation between speed of tumorigenesis and rate of development in different animals (p. 19).

In our own species there are definite racial differences. Thus the markedly lower incidence of cancer in exposed areas of skin in negroes and Hindus than in whites, and especially in fair-complexioned whites, which depends on the melanin in the skin preventing the penetration of the carcinogenic and mutagenic ultra-violet radiation, certainly has a genetic basis. Ward and Gunther (1954) find a somewhat greater proneness to skin cancer in native-born Australian whites (mainly of British descent) than in (non-British) immigrants. It would be of interest to determine the incidence in racial hybrids of cancers with different racial incidence. The markedly high incidence of skin cancer in albino Bantus (Shapiro, Keen, Cohen and Murray, 1953) has a single-gene basis.

However, careful analysis is usually needed to decide how much of these differences is genetically determined, how much is modificational (environmental). Thus in skin cancer light intensity also plays a part; in both negroes and whites in the southern United States the incidence of skin cancer is higher than in the north. The effect of strong sunlight is also shown by the fact, told me by Dr. E. B. Ford, that white-faced sheep show a much higher incidence of tumours on the face-skin in the North Island of New Zealand than in Britain. Sometimes the answer is clear. Thus, at first sight, the almost total absence of cancer of the penis in Jews might be taken to have a genetic basis, but has been shown to depend on very early circumcision. As Kennaway (1957) writes, 'the fact is now well established that cancer of the penis, excluding the meatus, has never been found, perhaps with one exception, in anyone circumcised by the Jewish method.' Moslems, who are circumcised up to 14 years of age, have a penis cancer rate which is lower than that of non-Moslems in the same area, but higher than that of Jews (Hieger, 1955, p. 27). In 16 cases of cancer of the penis arising in non-Jews in the

U.S.A. after circumcision at 13–39 (mean 23) years of age, the cancer developed after an interval of 11 to 39 (mean 23) years after the operation (Kennaway, 1957).

It is further probable that the rarity of cancer of the cervix in married Jewish women (and in virgins of all races, e.g. Canadian nuns; Hieger, 1955, p. 27) is to be partly ascribed to the same cause, smegma apparently having a carcinogenic effect (Oberling, 1952, p. 104), but partly to the Mosaic ritual called Niddah (Kennaway, 1957). Similarly, the very high incidence of liver cancer among Bantu Africans, Javanese, etc., appears to have not a genetic but a dietary basis (see Hieger, 1955, p. 24). This leads one to suspect that the high incidence of cancer of the digestive tract in Japanese men (double that of United States men) may also depend on dietary habits. The fact that in Japanese women breast cancer is six times as rare as in west European women may have some genetic basis, but its incidence certainly depends mainly on non-genetic factors, such as nursing habits and barrenness; in Europe, women of the wealthier classes show a higher incidence. The reason for the higher incidence of retinoblastoma in Bantu than in white babies is unknown.

We must also distinguish between complete genetic determination of cancer and cancer-proneness (susceptibility) of various degrees, though the two may grade into each other.

3. TUMOURS DUE TO GENETIC IMBALANCE IN WIDE CROSSES

Species (or wide subspecies) crosses often result in genetic imbalance and resultant abnormality of various kinds, for example, in *Antirrhinum* and *Lymantria* (references in Harland, 1936), so that we may expect them sometimes to cause genetic cancer by an upset of the balance between growth and its control. Accordingly, among the most striking cases of complete genetic determination of cancer are those in which hybridization of two normally tumour-free species results in tumours in all or many of the progeny. The best-analysed case is that of the plant genus *Nicotiana* (Kehr, 1951; Kehr and Smith, 1954). In twenty-six combinations between seventeen species, all the F_1 hybrids exhibited tumours, while in twenty other combinations of the same and two other species a moderate percentage was affected; a number of other inter-specific combinations gave no tumours at all.

The gene-complex of one species, *N. langsdorffii*, was particularly effective in causing tumours in crosses. Its cross with *N. glauca* has been especially well investigated. Reciprocal crosses gave identical results, showing that maternal cytoplasmic factors were not involved. So long as one complete genome of each species was present in the hybrids, 100% tumour-formation occurred, even when the proportion of *glauca* to *langsdorffii* genomes was varied sixfold, from 3: 1 to 1 : 2. The presence of one or a few only of the twelve *glauca* chromosomes in combination with two *langsdorffii* genomes gives no tumours; it will be interesting to see which and how many of the *glauca* chromosomes are responsible for causing the imbalance. The tumours, though they may be very large, are not fatal, but they inhibit meristematic growth in the branches where they occur, and predispose them to infection by rot. The general tumorous predisposition of the hybrid gene-system is shown by the fact that accidental exposure of leaves to a spray containing turpentine, whiting and white lead caused tumours at almost every spot of contact. There is an inverse relationship between rate of elongation and the appearance of tumours. When pieces of hybrid tissue, both tumorous and non-tumorous, are grown *in vitro*, they may be markedly affected by the conditions of culture; rapidly growing tumour tissue may have its growth rate slowed, or may be caused to embark on normal differentiation.

A similar case, originally discovered by Kosswig (1931), is found in fishes (Breider, 1940; Gordon, 1951, 1953). The F_1 of crosses between certain species of the subgenera *Platypoecilus* and *Xiphophorus* (and in one case within the former subgenus) regularly develop melanomas, which are often very large and may be fatal. Segregation in later generations produces forms with a great range of incidence and severity of the condition. Some gene-combinations are more tumorigenic than others (Gordon, 1954). No melanomas were found in over 20,000 wild specimens of either species.

The black pigmentation of normal *Platypoecilus* depends on two kinds of cells, the small micromelanophores and the very large macromelanophores; only these latter give rise to tumours. I have elsewhere (Huxley, 1955) made the suggestion that the major gene responsible for macromelanophore development in *Platypoecilus* also produces some deleterious tendencies, perhaps towards over-proliferation, but that these have been selectively buffered by modifiers during evolution; hybridization inevitably destroys the buffering system, and creates a genetic imbalance in which the deleterious tendencies of the main gene are no longer inhibited. A similar debuffering of a buffered pathological condition was found by Stockard (1941) in crosses between St. Bernard dogs, with their obvious acromegalic tendency, and Great Danes. Sometimes the particular combinations of genes responsible for melanomas (and for the one case of hybrid erythromelanoma, involving red as well as black pigment-cells) can be identified (Gordon and Nigrelli, 1950; Gordon, 1950). Humm (*in litt.*) and Humm and Clark (1955) find that the macromelanophores of melanomas in hybrid platyfishes show an increase of oxygen consumption (endogenous respiration) and of activity against tyrosine and 'dopa,' while the hybrid micromelanophores do not. Grafting experiments (Humm and Young, 1956) show that hybrid melanomas are derived exclusively from true macromelanophores.

An intergeneric cross between an albino *Xiphophorus helleri* ♀ and a black-pigmented *Molliensia sphenops* ♂ also gave macromelanophore melanomas, but of a different type, in the 'black' (black-pigmented) majority of F1 and succeeding generations (Elwin, 1957*a*, *b*). The blacks were large and heavily built and slow-maturing, as against the small (or even dwarf) rapidly maturing non-blacks, the males of which did not grow further after maturity. In later generations abnormal sex-ratios and apparent sex-reversal occurred, and some intensely pigmented specimens and others with deformed mouths appeared among the blacks. Many blacks (in 2 broods 18/40 and 11/15) developed melanomas, which were frequently fatal. The melanomas differed from those described by Gordon in the Platypoecilus-Xiphophorus cross (see above), in arising only at the base of the tail instead of in any position, and in not producing metastases. Further, they did not appear to be the direct cause of death, but rather a symptom of a marked endocrine imbalance associated with dropsy. The macromelanophore gene responsible was probably derived from the Molliensia parent. It appears that here too, debuffering of the macromelanophore gene has occurred, but has been effected via the endocrine imbalance caused by hybridization.

Elwin (*in litt.*) tells me that in one brood of 25 from the 2nd generation of backcrossing an F_2 intergeneric hybrid to *X. helleri*, 14 individuals developed fatal melanomas; these, however, did not show the marked restriction to the base of the tail, showing that this character depended on *Molliensia* genes. Unpublished work showed that an inbred line of another cyprinodont fish, *Heterandria formosa*, gave a high proportion of highly malignant melanomas. When the line was crossed with an unrelated strain of the same species, the production of melanomas was abolished.

The best mammalian case is the species-cross between the wild mouse, *Mus bactrianus*, and an inbred strain of domesticated *M. musculus* (Little, 1939). Here, though not all the F_1 developed tumours, the incidence was much higher than the sum of the parent incidence. The incidence of multiple tumours was increased about tenfold. Unfortunately, the hybrids are sterile, so that no further analysis could be made (Table 1).

TABLE 1. *Incidence (percentage) of tumours in a mouse species-cross*

Tumours	*M. bactrianus*	*M. musculus*, C.57	F_1
Epithelial	3·8	1·8	17·4
Non-epithelial	0·0	13·8	42·1

Woolley, Dickey and Little (1952) found a similar marked increase of various types of cancer in the offspring of a cross between two inbred strains of *M. musculus*. Intergeneric crosses in two families of birds resulted in tumours of the testis, in one case of interstitial tissue, in the other of spermatogonial tissue (Poll, 1920; Crew and Koller, 1936).

Species-crosses (including those in *Nicotiana*) may result in increased mutation-rate for some (or all?) genes (references in Harland, 1937). Since somatic mutation presumably plays a part in the genesis of certain tumours, this would reinforce the tumorigenic effects of genetic imbalance.

In interspecific crosses in the Jimson weed, *Datura*, ovular tumours occur. The endothelium (inner layer of the integument) proliferates to fill the embryo-sac; at the same time the growth of the embryo is inhibited. Rietsma, Satina and Blakeslee (1954) showed that the inhibition is due to an excess production of auxin (indole-3-acetic acid) by the tumorous tissue. These tumours (like crown-gall) are associated with excessive production of growth-promoting substances but are not malignant.

Seiler and Puchta (1956) found tumours or tumour-like growths among the F_1 of certain species-crosses in the moth genus *Solenobia*. These showed excessive proliferation of the proximal ends of the gonoducts, the ovarian tubes in females and the testicular ampullae in males. Seiler's suggestion is that their growth and differentiation is normally under some control by the germinal tissue: since this is absent in the hybrids, which are sterile and lack germinal tissue, uncontrolled growth occurs. In females the resultant proliferation is only moderate, but in males it may be great and result in huge tumour-like masses of unorganized tissue. These appear usually to be benign, though one had become invasive and was destroying the segmental musculature.

In the moth *Pygaera pigra* subspecific crosses show imbalance by producing all-male broods (Federley, 1936). In one cross only, all the offspring had lethal tumours. The tumours were in many sites and of many types, the commonest free-floating in the haemocoel. In various insects the three polar bodies of the zygote may fuse to give a triploid cell, which in some forms gives rise to functional embryonic tissue. Federley suggests that here a *Y*-borne mutation has reinforced genetic imbalance and caused excess multiplication of the triploid polar-body tissue, with consequent tumour-formation.

4. TUMOURS DUE TO CHROMOSOMAL IMBALANCE

Darlington and Thomas (1941) investigated the wild grass *Sorghum purpureosericeum*, which has a variable number (one to six) of supernumerary '*B*-chromosomes.' In the second pollen-grain division of plants with extra *B*-chromosomes these fail to divide and all pass to the generative pole. When there are three or more of them, over-large nucleoli are formed, mitosis is abnormal, and the pollen-grains die.

The *B*-chromosomes consist of heterochromatin. This appears not to contain major genes like the euchromatin, but to consist of so-called polygenes, and to be concerned with the regulation of general metabolic processes. In *Drosophila*, when euchromatic chromosegments are translocated into the neighbourhood of heterochromatic regions their contained genes often produce abnormal characters. In *Sorghum* the pollen-grains with large excess of heterochromatin behave as 'encapsulated tumours,' to use Darlington's phrase. Apparently the processes leading to protein synthesis are exaggerated; this, within the limited resources of the pollen-grain, prevents the competing processes underlying maintenance from functioning adequately, and leads to death. If the capsule could be removed and extraneous nutrients made available, we may suppose that proliferation would ensue and would produce a large tumour.

In *Antirrhinum*, a strain studied by Stein (1935), had a marked tendency to tumours (and also to excessive growth), though the precise genic mechanism was not analysed. Various cytological symptoms were produced, including the unique production of several hundred genomes in a single cell. One of Stein's figures shows a cell with ± 8000 chromosomes!

Here the occurrence of increased chromosome-number (polyploidy and polysomy) in mammalian tumours may be noted. This, like the frequent aberrations of mitosis in tumour tissue, is certainly not an initiating cause of tumours, but a symptom or resultant of their abnormal type of metabolism. However, it apparently does often lead to increased malignancy (see below, and Koprowski, 1956), as does the presence of fused V-shaped chromosomes in rat sarcomas (Yosida, 1955). This may be because, where a growth-regulating balance mechanism is involved, (*a*) most genetic changes are likely to impair the balance, and (*b*) only those which favour growth-promotion will survive, while those which favour growth-inhibition will become extinct through differential reproduction (cf. killer *Paramecium*, p. 86, and Sonneborn, 1954).

Among the various tumours, some of them polyploid, induced chemically in the field-vole *Microtus*, Muldal (1956) finds 'super-male' types, with two *Y*'s and often a 'dwarf *X*' (?), and 'female' types without any *Y*. In both, the ratio of the (heterochromatic) sex-chromosomes, *X* or *Y*, to the autosomes is increased.

Yosida and Ishihara (1956) induced a hepatoma in a rat by administration of *p*-dimethylaminoazobenzene. The hepatoma consisted of four tumorous masses, two in the second and two in the third lobe of the liver. They showed marked differences in ploidy, as seen in the table.

Tumour-mass	% of cells of different ploidy		
	2 *n*	4 *n*	8–32 *n*
1	29	52	19
2	75	18	7
3	40	38	22
4	96·6	3·4	—

Only No. 2 had distinct *V*-chromosomes. These observations suggest that the four tumour-masses were derived from different 'mutant' cell-strains produced during the development of the hepatoma.

5. CELL-POPULATION GENETICS OF TUMOUR STRAINS

Recent work on ascites tumours sheds new light on this problem (Hauschka, Kvedar, Grinnel and Amos, 1956; Amos, 1956; Makino, 1956; Hornsey and Howard, 1956; Richards, Walker and Deeley, 1956; Koller, 1956; Koprowski, Theis and Love, 1956), as part of what I may call the population genetics of cancer (Klein and Klein, 1956; Lederberg, 1956; Levan, 1956; and other papers in Miner, 1956).

The discovery that some solid tumours could be made to grow in ascites form as free cells or cell-groups in serous effusions in the peritoneal cavity made possible the accurate study of the variance of the cells of a given tumour strain, and the change of that variance with change of conditions.[1] The first point established was that tumour strains are not genetically homogeneous, but consist of cells differing chromosomally (in whole ploidy, aneuploidy, and structural peculiarities) and genetically (in regard to segregating mutations). The second was the validity of the stem-cell concept, that is, that the strain is reproduced by a small fraction only of these variants. Some variants are inviable, others cannot replicate themselves, others are at a marked selective disadvantage. The stem-cells can thus be regarded as constituting the tumour's potentially immortal germplasm, the remaining cell-types its mortal soma.

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[1] Several workers (see Kaziwara, 1954) have now succeeded in growing ascites tumour sub-lines from single cells. One polyploid sub-line from a modally hyperdiploid tumour strain remained unchanged for 20 weeks, but we would expect that all such cell-clones will eventually develop genetical inhomogeneity; it will be of great interest to discover the extent of such new variance and the rate at which it occurs.

Hauschka *et al.* (1956) have established that (in certain tumours at least) the development of near-tetraploid stem-cells enables the tumour to overcome a quite wide histocompatibility barrier (see p. 61) and so to survive when inoculated into mouse strains in which the original near-diploid tumour-cell population cannot be propagated. However, where the histocompatibility barrier is too wide, this 'immunoselection' through near-tetraploidy is impossible, the tumour strain remains diploid, and the tumours it induces in the alien mouse strains always regress. The capacity to grow in otherwise incompatible mouse strains depends on loss of antigenicity (and consequent failure of the alien mouse hosts to produce antibodies), apparently involving a change in the properties of the surface. The immunoselected near-tetraploid line had lost most of the invasiveness and virulence of the original near-diploid line, so that its survival in otherwise incompatible mouse strains must have been due solely to changes in its immunogenetic (histocompatibility) properties. Since malignant tissues usually have fewer histocompatibility requirements than non-malignant and normal homografts, it is suggested that this failure to produce certain antigens ('antigenic simplification') may be a general failure of malignancy, as well as induced by immunoselection. This latter will occur only in specific experimental situations, and is concerned only with histocompatibility requirements, while other imbalances may permit higher invasiveness, growth-rate, or virulence. See also Koprowski, Theis and Love, 1956.

Klein and Klein (1957) have shown that even a small number of histocompatible cancer cells injected into a monkey together with a large number of non-compatible cells will not only survive, but will survive better than if injected alone. This stimulating effect is exerted by cancer cells (of the same or different strain) lethally damaged by the host's immunity reaction or by X-rays, and may be intense: its precise mechanism is not yet known. Dead cells on the other hand totally inhibit the growth of a very small number of living cells of the same strain, owing to the development of powerful immunity reactions by the host (Révész, 1957).

In any case, it is not polyploidy *per se* that permits immunogenetic adaptation, but *genic imbalance*. A near-tetraploid strain can support greater variations in chromosome type (e.g. translocations) and number than a near-diploid one. Loss of whole chromosomes is commoner than gain, as shown by the fact that the modal chromosome frequency in the immunoselected tumour strain is hypotetraploid (Hauschka *et al.*, 1956). Levan (1956) has shown that in ascites tumours in general structural changes, notably translocations, are frequent, leading to an increased variation in chromosome-size, including the production of 'minutes' and abnormally long chromosomes.

The high variance of the tumour-cell population provides the basis for rapid selective adaptation. Thus in near-diploid tumours about 5% of the cells are near-tetraploid, and can eventually form the stem-line of an immunoselected strain. Conversely, the immunoselected near-tetraploid strain contains a small percentage of near-diploid cells; when the strain is returned to its original mouse host these are at a selective advantage owing to their greater invasiveness, and speedily become the stem-cells of a new near-diploid strain, with the same surface and antigenic characters as the original.

Koller (1956) finds that in some human ascites (natural effusions containing malignant cells) the malignant cells develop singly, in others in small colonies of up to eighty cells. The latter show a much higher degree of polyploidy, aneuploidy and mitotic irregularities. The colonies appear as syncytia, in some cases with nuclei of very high ploidy (cf. *Antirrhinum* tumours, p. 43).

In tetraploid cells of an ascites form of an ovarian teratoma, Griffin (1956) finds chromosome behaviour 'closely imitating meiosis,' showing synapsis, crossing-over, reduction and segregation.

Klein and Klein (1956) have clearly shown that the transformation of solid tumours to ascites type involves the selection of rare mutants, either pre-existing or arising during the experiment, from a variant cell-population (which attains numbers between 10^8 and 2×10^9 in each mouse individual), and not a gradual physiological adaptation of the entire population. In some cases, a succession of mutations appears to be involved. Furthermore, different tumours show different degrees of 'pre-adaptation' to ascites convertibility. The establishment of these facts, as Lederberg (1956) stresses, is of great importance not only for cancer research but also for general genetics and for epigenetic differentiation.

6. TUMOURS DUE TO SINGLE GENES OR SMALL SYSTEMS OF GENES

(*a*) *Drosophila*

Finally, we come to genetic tumours due to single genes or small systems of genes. The best known are the melanotic tumours of *Drosophila* larvae, though, as will appear later, it is possible that they are not true tumours (neoplasms) at all. Over twenty-five of these have now been identified, each caused by a different gene or set of genes, in various chromosomes. Many are caused by a single major gene, but some by multiple genes. The tumour-inducing genes are all completely or largely recessive, so that they are usually only detectable in inbred strains, as in mice (see later). Different genes determine different types, incidences and sites of tumours. Thus Burdette (1950) investigated nine inbred stocks which had shown spontaneous tumours. In standard conditions one strain had 1% tumour-incidence, another 50%. This second strain produced large abdominal tumours, while a third showed only small tumours in the head region. Tumour-incidence could be modified by population density, nutrition and temperature.

Modifiability of tumour-incidence has been reported by numerous other workers (references in Scharrer and Lochhead, 1950). In general, tumour-incidence is decreased by crowding and by higher temperatures, though the relation is sometimes more complex, and in one strain the opposite temperature-effect was found; in this case, two genes were involved, one of them being subject to a temperature-effective period limited to the first 24 hours of development. Arginine (but not ornithine) added to the standard diet increases tumour-incidence (in the strains tested). X-radiation may also affect tumour-incidence, lower dosages increasing and higher dosages decreasing it. Finally, as would be expected, the residual genetic background may markedly affect tumour-incidence (tumour-proneness).

The effect of crowding in reducing tumour-incidence has been analysed by Friedman, Harnly and Goldsmith (1951). It appears to be due to an exhaustion of the vitamins and other substances provided by live yeast in the larval diet, which are apparently required for the development of tumours. This was confirmed by the use of vitamin variations and analogues. See also Mittler (1954).

Wilson, King and Lowry (1955) maintain that the melanotic growths of *Drosophila* are only 'pseudotumours,' since mitosis never or rarely occurs in them, and their growth occurs by aggregation of blood-cells (Oftedal, 1952): one pseudotumour strain lacked cystine and an unidentified substance in its body fluids. Burton and Friedman (1956) and Friedman and Burton (1956), following Barigozzi, have demonstrated specific tumour-inducing factors in acellular larval extracts of most *Drosophila* tumour strains tested. See also Kaplan (1956).

Rizki (1956, 1957) agrees that they are pseudotumours, but concludes that they originate owing to a genetic timing error in the processes leading to metamorphosis. Their constituent cells are flattened blood-cells termed lamellocytes, which arise from the plasmatocytes which constitute the great majority of larval blood-cells. In normal development only a few lamellocytes appear during larval life, but during pupal life almost all the haematocytes become transformed into lamellocytes. In pseudotumour stocks (tu^w and mt^a) the transformation occurs precociously; in tu^w larvae the resultant lamellocytes encapsulate the caudal fat-bodies, and in mt^a larvae the remaining blood-cells. He was unable to find any evidence of increased cell-division in the blood-cells of pseudotumour strains. However, Ghelelovitch (1956), though agreeing that the bulk of the melanotic growth consists of a non-tumorous capsule of modified blood-cells, maintains that its centre originates as a mass of truly tumorous blood-cells. If so, the melanotic tumours of *Drosophila* are a peculiar type of benign neoplasm, in which the original neoplastic tissue is encapsulated (and in time apparently destroyed) by a large 'pseudotumorous' envelope of aggregated cells of other type, which then becomes melanotic through excess pigment-formation. But if, as seems increasingly likely, Rizki and the other proponents of similar views are correct, the growths are not neoplastic at all, but only pseudotumorous. The 'tumour-inducing' substances mentioned above would then be substances causing precocious lamellocyte transformation.

Whatever their origin, during metamorphosis the tumours are destroyed by histolysis like other larval tissues, and only inert masses of pigment remain in the imago. Oster (1954) ingeniously combined a tumour gene with another gene, 'giant,' which adds an extra moult and so lengthens larval life and doubles final larval size. The tumours then grew to a relatively as well as absolutely larger size, and were more frequently fatal, suggesting that if larval life could be indefinitely prolonged, malignancy might eventually supervene on a *de facto* benign condition. Ligation of larvae, by preventing the caudad spread of the pupation hormone, reduces the frequency of pupation in general, and may prevent it in the posterior end only. This increases the percentage of tumours in an inbred medium-tumour strain, from 15% in control late larvae to 43% in ligated larvae which failed to pupate, and 56% in non-pupated posterior halves, suggesting that either the pupation hormone or the histogenetic changes which it induces tends to prevent the formation or development of tumours. In this connexion it is interesting that Franks, Bather and Thompson (1950) found that the pupation hormone of blowflies (*Calliphora*) inhibited the carcinogenic action of methylcholanthrene on mice.

Plaine and Glass (1952, 1955) found that tryptophane fed to *Drosophila* larvae caused a large increase in the incidence of melanotic tumours, which was still further enhanced by prior keeping of the larvae in pure oxygen. They consider that the effective substance is formylkyrenunine or some other oxidation product of tryptophane, which inhibits two 'suppressor' genes, one that normally suppresses the tendency to tumour manifestation, and another which suppresses the manifestation of an eye-mutation, *erupt* (which itself is somewhat tumour-like, causing masses of hypodermal cells to erupt through the surface of the eye). Plaine (1955) further found that cysteine counteracted the effects of tryptophane.

King and Burnett (1957) have recently described a remarkable non-melanotic ovarian tumour in *D. melanogaster* homozygous for *fused* (fu). This sex-linked gene, among its numerous phenotypic effects, is now found to produce ovarian tumours and therefore female-sterility. In normal ovarian development (King, Rubinson and Smith, 1956) the cells in each egg-chamber arise from a single cell by 4 divisions, 15 of the resultant cells becoming nurse-cells for the 16th, which becomes an oocyte; after this no further cell-division occurs. The *fu* gene removes the inhibition on cell-division, and the resultant uncontrolled proliferation converts the egg-chambers into tumours containing hundreds or thousands of dividing cells. As King and Burnett suggest, it is probable that others of the 60 or so female-sterile genes known in *D. melanogaster* will prove to operate in a similar way. This discovery opens the door to much profitable research on the factors underlying uncontrolled proliferation.

One obviously malignant genetic tumour (depending on a sex-linked recessive lethal gene) has been discovered in *Drosophila* (Shatoury, 1955*a*). This arises from the over-proliferation of two of the three types of cell in the lymph-glands, which first invade the imaginal disks and then metastasize in the nerve-cord, fat-bodies, testes and gut. The case is unique in that a protective reaction is also induced; the third type of lymph-gland cell proliferates to encapsulate the tumour cells and in some unexplained way to cause their degeneration and melanization, leading to the formation of melanotic bodies similar to those formed by benign *Drosophila* tumours. Some of the abnormal behaviour of the lymph-glands in the tumorous strain appears to be an intensification of their normal behaviour. The tumours produced by Friedman and Burton (1956) by injecting preparations of an inherited tumour-inducing factor were invasive and lethal when purified preparations were used, but crude preparations induced only benign free-floating tumours. The purified factor appears to be or to contain nucleoprotein, and to resemble a virus in some respects (Friedman, Burton and Mitchell, 1957), and its activity is presumably partly inhibited by some other substance present in the crude extracts. Deprivation of various vitamins present in brewer's yeast leads to a slowing of larval development, and to a marked reduction in effectiveness of preparations of the tumour-inducing factor and in tumour-incidence in the *tu-e* strain producing that factor. The tumour-promoting activity of the vitamins was countered by vitamin analogues (Friedman, Harnly and Kopac, 1955).

Another sex-linked lethal (Shatoury, 1955*b*) causes abnormal proliferation of the imaginal disk mesoderm, resulting in failure of the imaginal disk to differentiate, and eventually in death. Shatoury suggests that this is the result of abnormal development of the lymph glands, leading to under-production of a hormone which normally regulates mesodermal proliferation. Here we have an example of abnormal proliferation on the border-line between hyperplasia and neoplasia, and of the usual conflict between proliferation and differentiation.

Two other half-way cases are reported by Rizki (1955) and Gowen (1934). Rizki found in *D. willistoni* eight non-allelic recessives which determined various patterns of melanotic spotting, all of them lethal, but none tumorous. Gowen obtained a sex-limited mutation (by X-radiation) which caused melanotic lesions localized in the femoro-tibial articulation of one or more legs. These arose just before emergence, and were not lethal until after a considerable time. The normal processes of pigment-formation, preparatory to pupation in the one case and in the other to emergence, appear here to have been deviated into lethal or sub-lethal channels, but without leading to growth-autonomy. Enzmann and Haskins (1938) claim to have obtained melanotic tumours, some of them very large and of extraordinary types, in certain sites in *Drosophila*, by X-radiation. These persist in the imago.

(*b*) *Mammals*

In mammals, the extraordinary case of grey horses deserves special mention (McFadyean, 1933; Raven, 1950). Grey horses (including the Royal Greys) are peculiarly prone to develop malignant melanomas. Whereas the total cancer incidence for old horses (over 15 years) in general is somewhere near 6%, that for grey horses is 80%. The incidence, of course, increases with age, but is abnormally high at all ages (Table 2).[1]

TABLE 2

Incidence of melanoma at different ages in 234 *grey horses*

Age (years)	<6	6–8	8–10	10–12	12–14	14–15	>15
Incidence (%)		11	36	61	71	71·5	80

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[1] Dr. Cotchin has pointed out to me that most of the melanomas of grey horses are in fact non-malignant and cannot be described as cancers. Thus, though genetic tumour-proneness is still striking, it leads primarily to the development of benign growths.

Epidermal melanin is more diffuse in black and brown than in grey horses. In greys melanin may occur in the cutis, apparently as a first step towards melanoma. The hairs of grey horses grow paler with age, often becoming white, while melanin accumulates near their roots. Melanomas may develop from such accumulations of pigmented cells. As the tail hairs are longest, the largest accumulations of melanin are near the base of the tail and the anal region is the commonest site for melanomas. Further study of this problem should be most rewarding. Why and by what mechanism does melanin accumulate at the roots of grey hairs as they become paler? Do pale grey breeds of other animals show the same phenomenon? Among horses, do dapple, flea-bitten and other types of greys (including non-albino whites, which in equestrian circles are classed as greys) show a different incidence of melanomas? Do melanomas occur frequently in the Lipizzaner horses in the Imperial Riding Stables in Vienna, which are born black and turn almost white as they develop (Fitzgerald, 1946; *Observer*, 1956), or in Percheron draught horses, which change colour in a similar way as they grow older? In piebalds and skewbalds, is there a higher incidence of melanomas in the white or pale portions of the skin?

Though man cannot be subjected to experimental breeding or treatment, he provides certain types of data not available for other species. Thus human twin statistics provide decisive evidence of genetic predisposition to cancer (Table 3; see also Busk, Clemmesen and Nielsen, 1948, and Verschuer, 1956). The cancers of monozygotic twins are generally of the same type and at the same site, and fre-

TABLE 3

General incidence of cancer in twins (after Darlington and Mather, 1949)

Type of twin	No. of twin pairs	Both twins of a pair affected (%)	Concordance of cancer type in affected pairs (%)	Interval in onset between members of affected pairs (years)
1-egg	62	62	95	7·5
2-egg, total	43	35	54	11·7
2-egg, like-sex pairs	27	44	58	10·8

quently become manifest at the same age. Table 3 refers to all types of cancer. The picture, as would be expected, becomes still sharper when only gastric cancer is considered (see Table 4), since we know from other evidence that marked genetic predisposition is here at work (p. 59): mammary and uterine cancer, however, show no difference in concordance between 1-egg and 2-egg twins, indicating that genetic predisposition to them is negligible (Verschuer, 1956).

TABLE 4

Incidence of gastric cancer in twins

(From von Verschuer, 1956.)

	No. of twin pairs, both affected	Cancer type in affected pairs		
		Fully concordant	Related	Discordant
1-egg	24	9 (37·5%)	2 (8·3%)	13 (54·2%)
2-egg	41	2 (4·9%)	10 (24·4%)	29 (70·7%)

Medical genetics, by analysis of human pedigrees, has also revealed something not yet demonstrated for other mammals—the existence of certain types of tumour completely determined genetically by single alleles. These include the recessive (and partially sex-linked) xeroderma pigmentosum and the semi-dominant (with effects in the hemizygous state) retinoblastoma and multiple intestinal polyposis (a condition which in nine cases out of ten becomes cancerous). References to these and many other examples are to be found in Kemp (1948), Woolley (1953), Gorer (1953), Snyder (1947) and Ford (1949).

Xeroderma pigmentosum is of particular interest. The skin of persons homozygous for this recessive gene inevitably develops malignant cancer—but only if exposed to light. The confusion of thought concerning the genetics of cancer is well revealed by Oberling (1952, p. 85), who writes 'to a superficial observer this could seem like a hereditary cancer, but what really is transmitted is not cancer at all but an excessive sensitivity to sunlight.' Of course cancer, like any other somatic character (including sensitivity to light!), cannot be transmitted; what is transmitted is a material self-replicating unit or particle in the chromosomes, which then determines or predisposes to the development of cancer in skin exposed to light. Heterozygotes show extreme freckling, unassociated with red hair. Two such persons should not marry, as 25% of their children are likely to suffer from xeroderma pigmentosum.[1]

I now come to the problem of genetic cancer-proneness—the genetic background which conditions the manifestation of cancer, by determining the degree of susceptibility to extraneous carcinogenic influence, or the incidence of genetically determined tumours (in genetic language, the *penetrance* of the genes concerned).

The importance of the genetic background for the manifestation of phenotypic characters is classically illustrated by the work of Harland (1936, 1937) on dark petal-spot in cotton (*Gossypium*). First, very similar *characters* (manifestations of spot) may, in different species, be determined by different alleles of one main gene. Secondly, the same allele which in one species will determine a typical large spot, will, against the genetic background of another species, produce only a small atypical one. In other words, the actual character depends on the interaction of a main gene and a number of modifiers which either inhibit its manifestation ('buffering,' p. 40) or enhance it.

In different species the modifier complexes appear to be adjusted to the 'desired' (selectively advantageous) result. In *Gossypium barbadense*, which is normally spotted, the modifier complex enhances the effect of the main spot allele, and therefore its degree of dominance; but in *G. hirsutum*, which is normally spotless, it does not do so, and reduces dominance. In species-crosses between spotted *barbadense* and spotless *hirsutum*, the F_1 showed only a medium-sized spot, and in F_2, in place of the sharp dominance and 3:1 ratio seen in intraspecific crosses, all grades of spotting appeared, showing that dominance had actually broken down. The property of mendelian dominance, indeed, has usually been *evolved* by a species or stock: modifiers are selected which drive deleterious mutants into recessiveness (Fisher, 1931).

A complementary result had been described in the moth *Triphaena comes* (see Ford, 1955). Here the apparently identical dark morphs of two dimorphic geographical races turn out to be determined by an identical dominant main allele, but with the aid of different modifier complexes, so that the dominance breaks down in crosses. Many other cases are known where the same gene will produce quite different effects according to the general genetic background.

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[1] Dr. E. B. Ford tells me that the frequency of this gene indicates that a balanced morphism may be involved (see Huxley, 1955), the heterozygotes possibly having a selective advantage in relation to the formation of vitamin D and the avoidance of rickets.

For the human cancer problem, work on mammals is naturally the most relevant; luckily the results are quite unequivocal. The pioneer work of Maud Slye, developed particularly by C. C. Little and extended by MacDowell, Strong and others (see, for example, Snell, 1941; Strong, 1942; Little, 1947*a*, *b*; Woolley, 1953) has demonstrated the existence in mice of large numbers of genes determining or affecting cancer-proneness for many types of tumour. Close inbreeding was used to build up a number of lines of high genetic purity (homozygosity). This revealed any recessive alleles which might be involved, and ensured the permanence of combinations of modifiers affecting the type and degree of cancer-proneness (susceptibility). Inbreeding, as Little writes, provides 'biological magnification,' and this leads to higher genetic resolution.

In the course of time, sublines of standard inbred strains often tend to diverge slightly in their characteristics, presumably as the result of the incorporation of different mutants affecting cancer-proneness, sometimes with the aid of unconscious selection. In one strain at the Chester Beatty Institute in London the incidence of cancer at 7 months increased from 10 to 50% in 6 years; in the Simpson sub-line PM an original moderately high incidence of bone tumours later decreased to zero. As would be expected, the crossing of two distinct strains followed by inbreeding and selection produces a number of sub-lines with varying incidence of quite different types of tumour, or with zero incidence of any tumours (Strong, 1929). Strong (1942) stresses the important point that, with any given type of tumour, genetic tumour-proneness usually runs parallel with ease of experimental induction of carcinogens.

The conclusion to be drawn from the work on mice is sweeping and simple: all types of mouse cancer investigated by adequate methods have some genetic basis. I have summarized some of the most striking results in Table 5; and see Shimkin's (1956) Tables V (mammary cancer), VIII (leukaemia), and IX (hepatoma).

TABLE 5

Genetic cancer-proneness in mice: (A) in different inbred strains, (B) with different single genes (from various sources)

	Incidence of tumours				
	Pulmonary	Mammary	Leukaemia	Others	Response of adrenal cortex to ovariectomy
A. Strain (and colour)					
A (albino) Bittner	90 %	Breeding ♀♀ 85 % Virgin ♀♀ 5 %	0 %	—	—
A (albino) Chester-Beatty	40 % (8 months); 95 % (12 months)	—	—	—	—
DBA (dilute brown)	—	Breeding and virgin ♀♀ 90 %	—	♀ gonad, moderate	Nodular hyperplasia
C3H (wild type)	10 %	100 %	0 %	Hepatoma, moderate	—
C57 Black	1 %	0 %	—	Gonad, very low	Negligible
C58	—	—	90 %	—	—
AK	0 %	0 %	60 %	—	—
Simpson sub-line PM	—	—	—	Bone tumours, originally 30–40 %, later 0 %	—
I	10–20 %	—	—	Stomach, subcancerous adenomatous lesions	—
CE (dilute)	—	0 %	Increased	♂ and ♀ gonad, high	100 % carcinoma
B. Genes					
A^y (yellow, recessive lethal)	Increased	—	—	—	—
d (dilute)	—	—	Increased	—	—
waved	Decreased	—	—	—	—
f (flex-tailed)	Decreased	—	Increased	—	—
hairless	Decreased	—	—	—	—

The comparatively few studies on rats have revealed similar genetic differences in cancer-proneness (Table 6).

TABLE 6

Genetic cancer-proneness in rats. From data of Dunning and Curtis (1952)

Strain	Incidence of tumours		
	Mammary	Adrenal	Bladder
A × C	64	4	5
August	58	22	11
Copenhagen	0	0	39

Crosses between inbred strains of mice have in some cases revealed the genetic basis in more detail. Thus Heston (1942*a*, *b*) demonstrated a multiple factor basis for proneness (susceptibility) both to spontaneous and induced pulmonary tumours. With mammary cancer, genetic proneness, as well as the Bittner milk agent (a virus; see p. 79) is of great importance. The relation between the genetic and the non-genetic factors involved has been analysed by Heston (1948), Korteweg (1948) and Dmochowski (1948) (see also p. 79, and Law, 1954). According to A. D. Timofeyevsky in Kiev (cited in Anon. 1956*c*), mouse fibroblasts in tissue-culture become neoplastic when both the milk agent and methylcholanthrene are added, but not when only one or the other is supplied. Fekete (1938) has made the important discovery that genetic high and low incidence of mammary cancer (in DBA and C57 black mice respectively) are mediated through differences in epigenetic (developmental) processes. In DBA mice the timing of cyclical proliferation and regression in the mammary gland is inaccurate, local groups of cells continuing in one phase of the cycle while most of the gland has embarked on the next phase: tumours arise mainly in cell-groups which show abnormal persistence in proliferation. Genetic imbalance is here expressed in developmental imbalance: where this does not occur, tumours do not arise.

Genetic differences in tumour-proneness are also revealed by the difference in type of tumour produced by different strains of mice in response to excess oestrogenic hormones (Table 7).

TABLE 7

Types of tumours appearing in mice of different strains subjected to oestrogenic hormones

(From Shimkin, 1956, after Gardner, 1947)

Strain	Mammary tumours	Testicular tumours	Lymphoid tumours	Pituitary tumours	Uterine cervical tumours
A	+++	+++	—	—	++
C3H	++++	—	+++	—	+++
CBA	+++	—	+++	—	+++
C57BL	—	—	—	+++	+
JK	—	++	±	—	?
PM	—	—	++	±	++
HE	++	—	±	—	++

The different response of the adrenal cortex of different strains to ovariectomy must also be genetically determined (Table 5; and see Shimkin, 1956, p. 175 and Table VII). What is upset here seems to be the endocrine balance. The CE strain reacts to ovariectomy by excess production of a 'male-like' hormone, the DBA strain by that of a 'female-like' one (Woolley, 1953; and see Woolley, Dickie and Little, 1952, 1953).

The introduction of single genes with known effects on other characters showed that some of these also influenced cancer-proneness (Table 5). Perhaps the most interesting is A^y, the 'yellow' allele of the main pigmentation gene of the albino series. This is well known as the first example of a lethal gene, being dominant in its phenotypic effect but recessive in its lethality, though exerting some deleterious effects in viability even in single dose; it increases the amount of yellow pigment in the hairs and decreases that of black, and promotes fat-deposition and increased body-weight.[1] It was later found that it increased proneness for lung cancer when introduced into low-incidence strains like C_3H (Heston, 1942*c*). Dilute (*d*) and flex-tailed

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[1] Body-weight and mammary cancer-incidence are also positively correlated (see Heston, 1942*c*).

(*f*) increase proneness to leukaemia (and also reduce general growth rate). It is to be noted that flex-tailed and other deleterious but wholly recessive genes *reduce* proneness to lung cancer.

Of course, cancer-proneness, like all characters, is also affected by environmental agencies. Thus in mice restricted diets reduce the incidence of most tumours, notably mammary cancers; low cystine diet reduces the incidence of methylcholanthrene-induced leukemia, while additional riboflavin reduces that of azo-dye-induced hepatomas (in rats); high temperature increases the incidence of mammary cancer, and isolation promotes their earlier onset (references in Shimkin, 1950). In man, many environmental and habitudinal effects are known (p. 115 f.).

In birds, Greenwood, Blyth and Carr (1948) have bred a strain of fowls extremely resistant to Rous sarcoma virus (and also to grafts of chemically induced tumours).

In man, Aird and Bentall (1953) established the important fact that the alleles of the *ABO* blood-group system markedly affect proness to stomach cancer. They originally concluded that *A* increased and *O* decreased the incidence of gastric cancer, and this was confirmed by other workers, e.g. Mayr, Diamond, Levine and Mayr (1956). However, Balme and Jennings (1957) have now shown that this only applies to cancers of the pyloric antrum, the reverse holding good for those of the body of the stomach. Aird and Bentall's conclusion was due to the fact that they were considering all gastric cancers irrespective of site, and the further fact that the majority of them occur in the antrum.

It had previously been considered that gastric ulcer proneness was increased by the presence of *O* and decreased by that of *A*. However, Balme and Jennings's figures show that this was due to a similar methodological error. Actually the incidence of ulcers in the pyloric antrum and body of stomach respectively is affected by *A* and *O* in precisely the same way as that of cancers; but the majority of ulcers occur in the body of the stomach. The $A/A + O$ ratios for the two sites are the same for cancers and ulcers—0·7 for the antrum, but only 0·45 for the body of the stomach. Thus the genetic effect of *A* or *O* is not concerned with predisposition to one or other type of lesion or disease, but with predisposition of one or other site in the stomach to both types of lesion. These facts will partly account for the occasional manifestation of proneness to familial gastric cancer in man.[1] In addition, there are other genes which result in familial inheritance of gastric cancer. The risk of getting gastric cancer among relatives of patients is over four times as high as in non-relatives (Anon., 1954). Proneness to gastric cancer in mice is stated by some authors to have a genetic basis.

However, as we would expect on general principles, the incidence of gastric cancer can also be affected by environmental factors. Doll (1956) finds that the 'equivalent average' ♂ death-rate per 100,000 (which is always much higher than the ♀) ranges from 48 for U.S.A. whites, through 76 for U.S.A. non-whites and 91 for England and Wales, to 209 for Iceland and 220 for Japan. Early in the century rural rates were usually higher than urban, but this difference has now disappeared. The nature of the soil may also affect incidence, peaty soils and iodine-poor (goitrous) regions showing higher incidence. High incidence is correlated with low socio-economic status: in England and Wales the incidence in the lowest of 5 socio-economic classes is nearly 2½ times as great as in the highest. In plantation labourers in Sumatra, gastric cancer accounted for 19% of all cancer cases among the Chinese, but only 1% among the Javanese. During the last 25 years, a marked decline in fatal gastric cancer has occurred in many countries, e.g. Norway, Holland and the U.S.A., while in others there has been no change, or, as in Britain, the decline is only just becoming apparent. Where a decline has occurred, it is more marked in younger people. Sufferers from gastric cancer show considerably lower incidence of atherosclerosis than do other patients.

As Doll says, no single hypothesis will fit all these facts, though it is almost certain that dietary habits as well as genetic constitution are involved. But the facts provide a number of clues, which research can now follow up. Armitage and Doll (1957) recall that, over a wide age-range, mortality from most types of human cancer rises approximately in proportion to a high power (about the 6th) of age. Mathematical analysis of the mortality data now indicates that this fact need not demand a corresponding number of mutational steps, as previously envisaged (Armitage and Doll, 1954), but can also fit a two-stage theory, in which the first step is due to a mututation causing increased proliferation (see above), and the second step is not the result of a continuous process of ageing, as envisaged by Platt (1955), but of a second mutational event.

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[1] The blood-group alleles appear to have no effect on the incidence of duodenal cancer. Indeed, the disease-proneness of the two sites is markedly different. Stomach ulcers fairly often become malignant, duodenal ulcers very rarely.

The most striking correlation of blood-group type with cancer-proneness has been recorded by Mayr, Diamond, Levine and Mayr (1956), who find that the frequency of persons with blood-group *A* in sufferers from pituitary adenoma is below 20%, as against nearly 40% in normal controls, whereas the corresponding figures for *O* are 60·2% and 45·8%. Mayr and his co-workers also draw attention to the puzzling genetic fact that, though *B* is the rarest blood-group in Europe and North America, it has not yet been found to be discriminated against in any pathological condition or to be at any selective disadvantage.

The increase of tumour-incidence in a cross between two species of *Mus* has already been mentioned. This (and other cases of genetic tumours resulting from hybridization) supports what may be called the imbalance theory of cancer—that all organisms need to evolve systems of growth-regulation and that cancers arise when imbalance in favour of growth passes a certain threshhold. As Little (1947*b*) writes, the life of a mammal is a constant battle to establish and maintain internal balance and control; the latent ability of cell-proliferation needed for repair and replacement is thus a menace. Little is speaking of somatic development in mammals; but similar balance systems must have been evolved in all other types of organism, and, as his own work demonstrates, are necessary on the genetic as well as on the somatic level.

The so-called histocompatibility genes determine resistance and susceptibility to transplantation (see Snell, 1952*a*, 1953; Borges and Kvedar, 1952; Law, 1954; Barnes and Krohn, 1957). Almost all the work has been done with tumour tissue, since this, with its rapid growth-rate, is more able to withstand the general resistance to homografts. Mice possess a wide range of such genes, some with large and others with small effects; and there can be no doubt that similar genetic diversity in compatibility occurs in other mammals. However, this genetic transplantation-proneness has no relation to cancer-proneness. Most early work was done with tumour tissue, since this, with its rapid growth-rate, is more able to withstand the host's resistance to homografts. It now seems clear that the histocompatibility genes detected by tumour-grafting are only a part of a larger system (containing certainly over 15 genes in mice, and doubtless similarly elaborate in other mammals), each determining an antigen capable of causing the breakdown of a homograft of normal tissue (Barnes and Krohn, 1957). Resistance to homografts, as shown by recent work (see Medawar, 1957, p. 143 ff., and references in Billingham, Brent and Medawar, 1956) is thus an immunological phenomenon; and its strength is genetically determined, resistance being absent in identical twins, and much reduced in the genetically closely related members of long-inbred strains. In unrelated individuals, the destruction of a non-tumorous graft is normally so rapid that differences in resistance caused by single genes are usually swamped, though their effects can readily be detected when the use of tumour tissue markedly lengthens the graft's period of survival. However, the histocompatibility genes differ markedly in the strength of their effect, and some, notably H-2, can cause the breakdown of skin exchanged between two isogenic strains differing only in respect of H-2, as rapidly as between unrelated strains (Counce, 1956). This is a reminder of the fact that degree of reproductive relationship is only a crude genetic standard, based on pre-mendelian concepts.

Snell (1952*b*) finds that the growth of tumour grafts can be enhanced by certain preparations of normal and tumour tissue, and inhibited by others: see also p. 114. Morgan, Morton and Guerin (1956) find that three malignant tumours of mice, after freezing at – 70° C., irreversibly lose their strain-specificity (but not their viability). This presumably involves alteration of their histocompatibility reactions.[1]

7. GENETIC CANCER-PRONENESS: GENERAL CONSIDERATIONS

The implications of the genetic analysis of cancer-proneness are clear. All large cross-breeding populations, including man, will contain large numbers of genes affecting cancer-proneness both positively and negatively in various degrees. We can assume that proneness to any particular type of cancer will show a wide degree of variation distributed in a frequency curve of more or less normal type, as has been shown for susceptibility to various drugs in mice and other animals (see Gaddum, 1933)[1] and to insecticides in beetles (Anon., 1956*a*). Since the genes affecting proneness to different types of cancer and susceptibility to different carcinogens will often and perhaps usually be different, the distribution of the general cancer-proneness of the population will resemble that of the lumped susceptibilities to a number of different drugs. The variation in response of mice of mixed strains to chemical carcinogens is very wide (Pybus, 1955).

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[1] Deep freezing may also lead to anabiotic dedifferentiation (p. 98).

This at once accounts for the lack of obvious 'heredity' in human cancer in general; inbreeding is needed to reveal the genetic basis of the many particular cancers involved. It also accounts for the fact that in occupational cancers only prolonged and 'overwhelming high intensity' of exposure will cause really high incidence (e.g. 75% for miners in radioactive mines, 100% for tar workers with over 40 years' exposure; Hueper, 1952); only the more genetically cancer-prone fraction of those exposed to lower intensities or shorter periods will develop tumours. Such phenotypic overriding of genetic diversity by extreme environmental agencies is of course a commonplace of genetics.

The same, of course, applies to other 'environmental' cancers, for example, lung cancers associated with smoking or with urban existence (Doll, 1955; Hill and Doll, 1956; Stocks and Campbell, 1955; Anon., 1956*b*; Hieger, 1955, ch. 4; Medical Research Council, 1957; and see p. 116). Some workers are still trying to dodge the issue by pointing out that not all those given to heavy cigarette-smoking develop lung cancer, and so it cannot be 'the cause' of the cancer. It is surely time that we should drop medieval concepts concerning causation, and think in terms of multiple correlation. We then find just what we should expect on the supposition (*a*) that some substances in tobacco smoke and in exhaust fumes or ordinary smoke are carcinogenic, and (*b*) that the population varies widely in cancer-proneness (susceptibility) to them. We find that lung cancer incidence increases, from low to high, from non-smokers through pipe-smokers, to moderate and heavy cigarette-smokers, both in urban and rural areas, but that the incidence is consistently higher for the urban population (Stocks and Campbell, 1955). We also find that, while the mortality from other cancers has remained fairly steady in Britain during the last 15 years, that from lung cancer has increased well over threefold, from about 5000 in 1940 to about 17,000 in 1955. However, to get 100% incidence, a far more intensive exposure to the carcinogenic influence would be necessary.

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[1] Dr. Gaddum informs me that much of this variation seems to be non-genetic. However, adequate genetic analysis of the differences in drug-resistance between different pure strains seems not to have been attempted; it should be of great practical as well as theoretical interest. On the other hand, Williams (1956), in his valuable work *Biochemical Individuality*, gives numerous examples of presumably genetic differences in response to drugs (ch. 8), as well as in various other physiological characteristics.

Blumberg, West and Ellis (1952) have shown that groups of patients with very rapidly and very slowly progressing cancers are psychologically distinguishable (e.g. by the multiphasic personality inventory test), and that there is a significant correlation between certain readily measured psychological factors and neoplastic activity. Given the genetic basis for psychosomatic differences, such correlations are to be expected. It would be valuable to make a systematic survey of the incidence of various cancers in different somatotypes (Sheldon, 1940, 1954), and also in various types of insanity and nervous disorder.

8. GENETIC CANCER-PRONENESS: EVOLUTIONARY CONSIDERATIONS

Wherever genetic differences already exist in a strain, or come into being by mutation, natural selection will operate. According to circumstances it may operate to promote or to prevent change, to increase or to reduce variability, or to maintain a morphic (polymorphic) balance, as with human blood-groups.

One common effect I have referred to—the evolution of dominance, rendering favourable mutants more dominant, unfavourable ones more recessive. This latter change will only operate when the unfavourable mutation is fairly frequent, and when its effect is not too extreme to be readily buffered by modifiers. We should thus expect most mutations determining cancers or increasing cancer-proneness to be recessive, as appears to be the case. Such as are semi-dominant (p. 53) are presumably rare.

Selection has always operated to keep mutation-rate down, since a high rate would not only introduce too many deleterious alleles into the gene complex, but would also threaten the permanence of advantageous modifier complexes and other gene-combinations, and endanger the stability of the species. The excess mutation seen in some hybrids (Harland, 1936) is presumably due to the breaking down of the specific mutation-limiting complexes concerned, and may perhaps be responsible for some of the excess tumours produced.

When selection is very intense, surprising results may be achieved. Ford (1949) mentions the low incidence of cancers in the mucosa of the human nose. This may be correlated with the fact that it is more subject to chemical irritations and extremes of temperature than any other non-squamous epithelium; and that accordingly specially high resistance to tumorigenic agencies would be selected for. However, even this resistance can be overcome by high intensity of carcinogenic stimuli. Thus the Bantus' excessive snuffing of tobacco leads to a considerable incidence of cancer in the nasal mucosa (Keen, De Moor, Shapiro and Cohen, 1956).

Reverse mutation has been found for most genes. Its apparent absence in tumours is presumably due to its undetectability. Reverse mutation in a tumour would lead to lower proliferation-rate or invasive capacity; and the mutant cells would be speedily outgrown by the rest.

Mutations occur in somatic as well as in germinal tissues. Accordingly cancer strains which can be reproduced by grafting are capable of evolutionary change. The resultant change is almost always towards increased virulence—higher growth-rate, greater degree of malignancy. This is to be expected; once a tumour has become autonomous, it is automatically subject to a form of orthoselection, in favour of still further autonomy.

When tumours are artificially introduced to new types of host, for instance by grafting, they either fail to take (become extinct) or become adapted to the new environment. This may be so different that the tumour can only persist in a different type of host tissue; metastases from tumours in alien hosts may prefer quite new sites.

Viruses in general are known to be capable of very rapid evolutionary changes ('adaptations'), based on selection of new mutants. When parasitism is involved, as with phages in bacteria, parasite infectivity may be adjusted to host resistance so as to give optimum stability to the relationship. This has occurred, for example, in lysogenic strains of bacteria. This balance may be upset by viral mutations for increased virulence, and the upset then corrected by bacterial mutations for increased resistance. This dialectic process may continue through a number of such double steps (Hewitt, 1954*a*, *b*).

Whenever specialized modes of viral transmission occur, as with the Bittner milk factor in mice, or insect-transmitted plant viruses, we can be sure that they are the outcome of a long evolutionary adjustment. Tumour viruses, too, require adaptation to alien hosts, and achieve it by methods similar to those found in tumours—increased (stepwise) virulence with repeated passage, and utilization of new sites for attack (pp. 76, 80).

Finally, selection will tend to delay the onset of deleterious effects with a genetic basis, as far as is possible; for individuals with delayed onset will on the average be able to beget and rear more offspring. By these means, the manifestation of certain genetic defects may be driven into the post-reproductive period (for a general discussion, see Comfort, 1954). This is not possible for malignant conditions with very early onset, such as leukaemia, 85% of which occurs in childhood: no reproductive advantage would accrue if the age of onset were delayed from early to late childhood. It also is not possible with very rare mutants; selection-pressure favouring delayed onset increases with increased frequency of the mutant.

In man, carcinomas as a whole occur late, with a peak around the end of the reproductive period in women and at a corresponding age in men; while almost as many sarcomas occur in youth or early maturity as in late middle or old age. Further, carcinomas are much commoner than sarcomas. Ford (1949) suggested that this was due to selection delaying the average onset of carcinoma more than that of sarcoma. In early human societies few individuals could be expected to pass the age of 40 or 45, and even under the Roman Empire the mean expectation of life was only about 30, so that carcinoma-proneness could not be unmasked as a not uncommon condition of the menopausal and post-reproductive period until the expectation of life had increased. The gene-combinations determining sarcoma-proneness are so much less common that selection-pressure will be too feeble to effect much delay in onset.

This view of Ford's, as repeated later by me (Huxley, 1951), has been attacked by Dorn and Cutler (1954).[1] Thus they say that the magnitude of the incidence-rates they figure for different tumours bears no consistent relationship to the shape of the curves for incidence by age. Considering that almost all of those figured are sites for carcinoma, this is irrelevant; the main point at issue is whether general average differences exist in regard to frequency and time of incidence as between sarcomas as a whole and carcinomas as a whole. Of course, if specific time of incidence is very early, it will be impossible for selection to delay its onset until the post-reproductive period. When incidence is very early, as in xeroderma pigmentosum (p. 53), an alternative evolutionary method of palliation is for the deleterious effects of the gene to be rendered recessive. This has not occurred with genes for tumours with late incidence like multiple intestinal polyposis.

Then, while admitting that the relative frequency of sarcoma decreases after adolescence, they argue that its incidence-rate later continues to increase very similarly to that of carcinoma. This again is irrelevant. It is likely that the age-incidence curves for most types of cancer will have a somewhat similar shape. The point at issue is whether selection has shifted the peak incidence to a later age. Finally, they seek to dispose of the matter by the statement that the incidence of cancer of a specific site appears to be determined largely by factors peculiar to that site itself. This last statement involves the same kind of fallacy as that in the question whether heredity or environment is the more important—to which the answer is of course neither, for both are always essential. Certainly age-incidence curves will show site-specific features; but this does not exclude the fact that they may also be modified by selection.

Theoretically, it is inevitable that selection should exert a delaying

[1] This paper and Dorn's (1955) contain much valuable information on the age-incidence curves, and also on the geographical incidence of different types of cancer in the United States. The age-incidence of different tumours may differ widely. For an analysis of age-incidence in breast cancer see McKenzie (1955).

effect on common and moderately late-appearing cancers, just as it is inevitable on theoretical grounds that a selective balance should exist between the members of a polymorphic system like the blood-groups (see Ford, 1956).[1] If a selection experiment were carried out on a strain of mice, selecting one sub-line for early onset of some type of cancer, another for late onset, with a third as control, I would prophesy that marked changes in mean time of onset would be achieved.

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[1] The theoretical inevitability of this latter result was pointed out long ago by Fisher (1930). It took over 20 years before medical scientists accepted this conclusion, or indeed took the trouble to check it.

B. Viral and Epigenetic

I

CARCINOGENIC VIRUSES

1. INTRODUCTORY

A NUMBER of tumours are caused by viruses, in the sense of minute (filterable), replicable, and transmissible (infective) particles in the cytoplasm. Different viruses can produce a number of different types of pathological change in their host cells (as well as, in some cases, remaining inactive—masked, latent, etc.). Even less is known about the precise means by which tumour viruses produce the metabolic deviation leading to tumour-formation than about the tumorigenic changes induced by X-rays and chemical carcinogens (see p. 101). However, the fact that they must primarily affect the cytoplasm is important, and points the way to further research. The study of non-tumour viruses is throwing light on the nature and mode of action of tumour viruses, while that of viruses in general (and of other paragenes, see p. 72) is illuminating some basic features of biology.[1]

Rous in 1911 showed that a certain type of fowl sarcoma could be induced by the transmission of a filterable agent or virus, consisting of particles of characteristic size, capable of infection and of replication in new host cells. As the notion of virus infection did not fit in with current ideas on cancer, this fact was played down for a considerable time; fowl tumours were usually dismissed as being in some way irrelevant to mammalian and human cancer. However, with the discovery of other virus tumours and the rapid progress of virology in general, much attention was given to the role of viruses in tumorigenesis, notably by Gye. Some authors, indeed, like Oberling (1952), go so far as to maintain that all tumours are due to viruses, the fact that in most cases no separately transmissible (infective) agent can be found being ascribed to its being in a 'masked' or 'latent' state.

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[1] Oberling and Guérin (1954) give a general review of the role of viruses in the production of cancer.

Such a theory, by claiming too much, becomes misleading. However, the fact that tumour agents (and other viruses) can in certain conditions become 'masked' (pp. 75 f, 84), together with new knowledge about viruses in general and about plasmagenes and symbionts, are leading us towards a truly central problem of organic life—the relation between genetics, development and infection. As Darlington and Mather say (1949, p. 215), cancer research lies at the meeting of the ways.

2. VIRUSES AS ONE TYPE OF PLASMID (PARAGENE)

Some of the ideas emerging from the vast volume of recent research are as follows. (1) Replicating units other than nuclear genes but containing nucleo-protein are involved in various aspects of metabolism in organisms.[1] (2) The replicating units may be intrinsic, originating as part of the organism's replicating system; or extrinsic, originating as independent entities which later intrude into the organism. (3) The resultant metabolic activities may subserve the whole system, or be deviated to the advantage only or mainly of the replicating units.

Such replicating units can be of very diverse nature—plastids, plasmagenes, viruses, units like Kappa particles in *Paramecium*; even alien bits of desoxyribonucleic acid (DNA) can be artificially foisted into the replicating organization of certain bacteria (p. 85). We need a neutral but comprehensive term for all such units; I suggest *paragenes*,[2] as implying that while they are not true nuclear genes, they resemble them in certain ways. I shall continue to use the term *virus* in its customary sense, as denoting a transmissible agent of submicroscopic size, capable of multiplication only within living cells, though viruses as thus defined are as heterogeneous an assembly as vitamins.

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[1] It is not certain that such units are always *self-replicating* in the customary sense; they may be normally *replicated* as a result of a complex set of metabolic processes, in which they play an essential role; see pp. 74, 92.

[2] Lederberg, in a brilliant review (1952), has suggested the term *plasmid* for all 'extra-chromosomal hereditary determinants,' including plasmagenes, proviruses, certain viruses and symbionts. The only reason I have not adopted this term is that it is confusingly similar to *plastid.*

3. PROPERTIES OF TUMOUR VIRUSES

The wide range of tumours induced by filterable agents (viruses) include in plants the wound-virus tumours (see p. 33; Smith, 1954, p. 83), in fowls the Rous sarcoma, angiosarcomas, myxosarcomas, endotheliomas, and avian leukosis (erythromyeloblastosis); in mammals the Shope papilloma and myxoma of cottontail rabbits, and certain mammary cancers of mice; and in frogs the Lucké renal carcinoma.

(*a*) *Shope papilloma and avian leukosis*

The two agents differ considerably in their properties (Beard, Sharp and Eckert, 1955) (see Table 6). In addition, the avian leukosis agent has a high phosphorus (phospholipid) content, and is one of the few viruses which contain an enzyme (adenosinetriphosphatase). As an integral part of its organization, constituting the infectious entity, it also contains three viral antigens, including Forsmann antigen and 'one indistinguishable from normal chick host-tissue.' These last two Beard considers to have been incorporated from the host into the developed virus particle.

TABLE 6

Properties of two viruses

Virus	Hydrated density	Dry density	Water content (%)	Fat	DNA	Structure	Size (mean)	Malignancy
Shope papilloma	1·133	1·31	58	Low 1·5%	8·7%	Rigid (resistant)	65·6 $m\mu$, uniform	Not immediate
Avian leukosis	1·059 (low)	1·29	80	High, ?30%	Lower	Non-rigid (fragile)	120 $m\mu$, variable	Immediate

It is notoriously difficult to be sure that one is dealing with 'pure' virus preparations; or, to put the matter differently, whether various substances found in a given virus preparation are essential (intrinsic) constituents of the virus, and if so whether constant or variable; whether they have been incorporated from the host; or whether they are inessential accretions or accidental impurities. Indeed, since a virus may be regarded not as a distinct entity like a bacterium or an animal species, but as part of a joint metabolic system, one of whose results is the synthesis of more virus in the host cells, the 'composition' of the virus, notably in regard to proteins, may vary according to the stage of the virus cycle and the physiological state and genetic properties of the host (Pirie, 1956). Thus, analyses such as those of Beard and his associates may need modification in the light of further research. Already Bender and Parkinson (1956) have developed a new method for isolating viruses which gives rather different results as regard virus size and composition. 'Masked' leukosis virus occurs in some strains of birds (Eckert, Sharp, Beard and Beard, 1952).

Beard inclines to the view 'that the [leukosis] agent originated initially from the host cell . . . developing with time the properties of autonomous existence and the newer structure unique to the agent.' It has taken almost fifty years to clarify the situation for avian leukosis, though its virus is abundant in the plasma; Beard suggests that many apparently non-viral tumours may eventually be shown to have a viral basis.

Recent work (Green and Beard, 1955; Eckert, Beard and Beard, 1956) indicates that avian leukosis is really a complex of diseases, caused by a number of distinct but related viruses.

A further major difference between the two types of virus is that in avian leukosis malignancy is 'the first evidence of infection,' whereas in the Shope papilloma it is not, supervening only late and only in some infected areas (warts).

The Shope virus (Beard *et al.* 1955; and see Oberling, 1952) occurs naturally in the wild cottontail rabbit (*Sylvilagus*) of America. Here it causes large papillomatous warts, which may either be resorbed, or persist, or in a minority of cases become malignant. They can be induced by cell-free filtrates both in other cottontails and in domestic European rabbits (*Oryctolagus*). The virus particles are said to be morphologically indistinguishable from those of human benign warts.

In *Oryctolagus* the warts grow much faster, and most eventually become malignant, but no virus (filterable agent) can normally be extracted from them (or from *malignant* cottontail tumours), though small tumours can be obtained with extracts from them applied to skin sensitized by repeated painting with carcinogens or irritants. Serologically both papillomas and carcinomas elicit antibody formation, though only for a limited number of graft passages. There is great variation in infectivity of virus from different cottontail warts. Beard *et al.* showed further that there was a wide variation in (genetic) susceptibility to infection with purified virus; after a certain time, acquired resistance reduces susceptibility.

The closeness of association between virus particles and host cytoplasm can vary greatly. The virus is more readily liberated from slow-growing benign tumours. Increased growth-rate and malignancy increase its 'masking'—i.e. the closeness of its association with host tissues—and diminish its freedom. This would seem to be the result of a long evolutionary adjustment of virus to host, too great virulence being disadvantageous to the virus. We find similar adjustments in parasites; thus the same trypanosomes which live harmlessly in the blood of wild game in Africa cause fatal nagana disease in domestic cattle and horses. It should be mentioned that Beard (1956) finds that virus 'masking' (pp. 75 f, 84) may be due solely to low virus concentration. However, this important finding cannot apply to prophage (p. 85), and it still seems probable that some examples of loss of infectivity in viral cancers are due to a true masking of the virus, by its close association with host elements.

(*b*) *Rous sarcoma*

The Rous sarcoma (see Oberling, 1952; Rubin, 1957) can be transmitted by cell-free filtrates, and by dried or glycerol-stored material. Rubin (1955) finds that one virus particle suffices to initiate tumour development. On chick chorioallantoic membrane, virus of the same strain can initiate ectodermal or mesodermal tumours according to the type of tissue infected. The virus does not destroy the host-cells as with lysogenic bacteria, but enters into a permanent stable association with them.

The virus normally causes tumours only in fowls. But it can be made to 'take' in other species, such as pheasants, guinea-fowl, pigeons, ducks and turkeys, by repeated passage through very young birds (see Borges and Duran-Reynals, 1952; Duran-Reynals, 1956). This increases the malignancy, as does repeated passage through a relatively resistant strain of fowls. In turkeys, while young chicks can be readily infected, birds after three weeks of age are refractory. However, Harris (1956) finds that injection of day-old chicks with whole blood or red blood-corpuscles from uninfected fowl chicks confers a persistent acquired tolerance of Rous sarcoma on them, implying that the virus contains a host component. This is confirmed by the fact that with repeated graft passages in turkeys, the infectivity of the virus for fowl chicks decreases markedly; but if the virus is successfully returned to fowl chicks, one passage suffices to restore its full infectivity for fowls.

The virus can grow even in mammals, but only in the anterior chamber of the eye. Here the virus develops into a form which, when returned to fowls, causes periosteal tumours (never produced by normal Rous virus). Alteration of the virus was also found in ducks (the most resistant of susceptible alien bird species). In 'immediate' duck tumours, developing within four weeks of inoculation, the virus was still of fowl type, and the tumours could not be readily transferred to further ducks. But that from 'late' tumours, developing only after several months, was changed to a duck virus; the tumours were returnable to fowls only with difficulty, but readily propagated in ducks, where they produced new types of tumour, including osteosarcomas and leukaemias. Clearly mutational evolution of different types may occur in the virus in an alien host.

Tumours usually take longer to appear after inoculation with cell-free filtrates than after grafting, apparently passing through a non-infective phase of multiplication of disaggregated sub-units (Carr, 1953). The Rous sarcoma is not contagious or infectious as between birds in the same pen. Tumours grow better and are more malignant in young and healthy birds; in old birds, it is almost impossible to induce tumours with filtrates, and grafted tumours show 'masking' of the virus, so that infective filtrates cannot be obtained. In embryo (or new-hatched) fowl chicks, inoculation of the virus causes, not tumours but haemorrhagic disease (see Lo and Bang, 1955); and in tissue-cultures of chicken fibroblasts, not increased proliferation but cytopathogenic changes (Lo, Gey and Shapras, 1955). Some types of viral fowl lymphomatosis are neoplastic, others not (see Duran-Reynals, 1956, p. 419). It is not infective to old birds, but highly so to young ones (some of which develop endotheliomas instead of lymphomatosis), though with malignancy developing only after several months' latency.

When tumours are induced by injection of Rous sarcoma virus, the amount of virus extractable from them varies with the amount injected: when the amount injected is very small, tumours will still be produced, but 24% contain no extractable virus. Hydrocortisone and the non-antibiotic bacterial product xerosin (which beneficially modifies the symptoms caused in mice by influenza virus), when injected together with dilute Rous sarcoma virus, lead to a much prolonged latent period and to atypical (hard and non-invasive) tumours. If xerosin is not administered until the tumours become visible, they grow more slowly and become atypical: but hydrocortisone administered at the same stage caused more instead of less rapid tumour-growth (Groupé, Rauscher and Bryan, 1956).

(*c*) *Lucké carcinoma*

The Lucké adenocarcinoma of the kidney in adult frogs, *Rana pipiens*, has a viral agent (Briggs, 1942; Rose, 1952; Rose and Rose, 1952). Briggs obtained interesting results with grafts into tadpoles of the same subspecies. In general, grafts into larvae succeed better than into adults, where they only grow well in the anterior chamber of the eye. All grafts in the tail ceased growing and regressed shortly *before* the onset of tail-resorption at metamorphosis. This is one of the indications that the substances causing tissue-resorption, which must be produced during metamorphosis, are inimical to tumour growth (see p. 114). It would be of interest to determine what happens in thyroidectomized or otherwise neotenous tadpoles; presumably resorption would not occur.

Grafts in other sites were not so successful. In several cases where tumours failed to develop at the site in the larva, multiple tumours appeared in the kidney, but only some time *after* metamorphosis (see Tweedell, 1955). The suggestion is that a virus was liberated, with a strong affinity for adult (but not larval) kidney tissue. Lucké had previously found that grafts into adult eyes resulted in an increased incidence of renal tumours (which did not appear to have originated by metastasis).

Rose and Rose (1952) succeeded in grafting Lucké carcinoma into urodeles. When returned to *Rana pipiens* it showed affinities for skeletal as well as for renal tissue (cf. the alteration of the Rous sarcoma agent in other species of birds). Rose (1952) and Tweedell (1955) also found alterations of the agent when grafts were made into other subspecies of *R. pipiens*. After repeated passages, the virus in some such cases does not produce characteristic tumours, but lesions 'causing much more histolysis than hypertrophy.' Sometimes, however, new types of tumour are induced. In one case a tumour in the iris proved to have acquired double specificity, for iris as well as for renal tissue; it is suggested that the virus may acquire new properties by combining with some component of a new site. Certainly tissue (site) specificity is very high; when grafted tumours develop in new sites, secondaries often appear only in the precise contralateral site. These results, if confirmed, should throw much light on viral tumour agents.

(*d*) *Mouse leukaemia and Bittner milk agent*

Gross (1951, 1954), S. E. Stewart (1954, 1955), Law (1955), Woolley and Small (1956) (and see Latarjet, 1957), have shown that some types at least of mouse leukaemia are due to a filterable virus, which, however, is transmissible through the egg (cf. p. 87). Inoculation of cell-free filtrates from high-incidence strains into newborn mice of low-incidence strains, or repeated passage through hybrid strains, causes not only leukaemia but parotid gland tumours, in C3H but not in C57 Brown mice (Gross, 1955, 1956; S. E. Stewart, 1954). McDowell (1955; and see Graff, 1956) has recently found that the milk of old mice (of certain strains only) contains a substance antagonistic to the development of leukaemia.

Recent work (Gross, 1957) indicates that several related viruses, or distinct forms of the same virus, may be involved in mouse leukaemia, and may produce different symptoms (cf. avian leukosis, p. 74). The filtered extracts from high-leukaemic mouse strains such as Ak and C58 will produce various forms of leukaemia after a latency of 3 to 18 months when inoculated into newborn (but not adult) mice of non-leukaemia-prone strains like C3H and C57 Brown. The susceptibility of different substrains (of C3H mice) varies greatly. The diameter of the transmissible agent (virus) particle appears to be less than 70 mμ.

Furth, Buffett, Banasiewicz, Rodriguez and Upton (1956) have also induced increased incidence of leukaemia (and increased mortality without leukaemic symptoms) by injecting newborn mice of low-leukaemia strains with tissue extracts from a high-leukaemia strain. Many of the induced leukaemias were of different type from those characteristic of the donor strain; and some of them had the immunogenetic properties of both donor and recipient strains. The authors make the interesting suggestion that this last fact may be the result of a process analogous with the DNA-induced transformation of bacteria (p. 85) rather than of ordinary viral infection.

In a preliminary note, Benedetti and Bernard (1957), claim that the particles they have discovered by electron microscopy in the spleen and bone-marrow of fowls suffering from erythroblastosis are carcinogenic viruses. They have found them also in normal 9-day and 15-day embryos, which indicates hereditary transmission via the egg cytoplasm, while their rare occurrence in healthy adults would imply the existence of a masked phase. Further data will be awaited with interest.

One of the most interesting tumour viruses is the well-known Bittner milk factor in certain mammary cancers of mice (see Greenstein, 1954, ch. 4; Oberling, 1952, p. 175). It is normally transmitted in the mother's milk, so that foster-nursing alters tumour-incidence. Thus, in one experiment, incidence in animals of a high-incidence strain possessing the milk agent was reduced from close on 100% to below 50% through fostering by agent-free mothers; and, in another, incidence in a strain without the milk agent was raised from 1 to 82% by the reverse process (Andervont, 1945). It appears that tumour incidence depends on a combination of genetic cancer-proneness and milk agent (p. 57; and Law, 1954). The infective efficiency of the virus decreases markedly with age of host. Thus, in a high-incidence strain, infection immediately after birth caused nearly 100% of tumours (of course many weeks later), while the incidence was reduced to about 60 and 10% in mice infected at 20 and 120 days respectively (cited by Duran-Reynals, 1956). In high-incidence strains both genetic proneness and the milk agent are involved; but some low-incidence strains lack the milk agent. Others, on the contrary, are so tumour-refractory that fostering with milk containing the agent will not cause tumours, though the agent remains active in their milk and can induce tumours in other strains.

The agent may sometimes be transmitted by the males' sperm as well as in the females' milk (Bittner, 1952). Presumptive virus particles have frequently been reported in various tissues, and recently Graff (1956) has isolated and photographed the virus. It has been suggested that the virus is present in all strains of mice, but permanently 'masked' in some. This, however, would not explain what causes the masking, and why no milk agent exists in rat mammary cancer, nor why it has so far proved impossible to induce cancer, in mice fostered on agent-free milk, by cell-free inoculation or injection.

(*e*) *General considerations*

Out of his wide experience Duran-Reynals (1956) emphasizes the following points, which are common to most tumour viruses that have been thoroughly investigated.

(1) Infection is much easier in embryos or very young animals, either of the same or of different species.

(2) The same virus which normally causes tumours may, in very young animals, produce haemorrhagic or other non-neoplastic disease.

(3) Strain- and species-specificity of a virus may be altered by adaptation to a different strain (mice, leukaemia), subspecies (frogs, Lucké carcinoma), or species (birds, Rous sarcoma, lymphomatosis; rabbits, papilloma).

(4) This often results in a change in site-specificity and in type of tumour induced.

(5) By repeated passages on solid media latent viruses may become 'unmasked' (e.g. Huebner (1954) recovered adenoidal-pharyngeal-conjunctivitis virus in 4% of standard cultures, but in 57% after frequent passage).

Duran-Reynals points out that most of these facts can be paralleled with non-tumour viruses. Thus *ad* (1), many viruses can be best detected by inoculation of embryos or newborn animals; *ad* (2), 'pox viruses may stimulate or destroy cells, under different circumstances'; *ad* (3), rabies virus may be adapted to rabbits; *ad* (4), some influenza virus strains may become neurotropic; *ad* (5), we have the conversion of prophage to phage by ultra-violet (p. 85).

He suggests that the inoculation of tumour cells or filtrates into embryonic or very young hosts, of the same or related strains or species, would be expected to reveal the presence of viruses in many cancers now considered as non-viral; the diagnostic symptoms would be (*a*) the speedy appearance of non-neoplastic lesions; (*b*) the later appearance of tumours, especially those of new type.

II

OTHER VIRUSES

1. TYPES OF VIRUS

OTHER viruses fall into various main categories: higher plant viruses, insect viruses, vertebrate viruses, bacterial viruses or phages, and rickettsias (see, for example, Smith, 1954; Burnet, 1955). As regards nucleic acids, the plant viruses (see Smith, 1955*a*) contain only ribosenucleic acid (RNA) (perhaps because they mainly infect non-dividing tissue; Bawden and Pirie, 1952); most others contain mainly or solely DNA, though the main nucleic acid component of influenza virus is RNA. The rickettsias are intermediate in size and complexity between other viruses and bacteria, and are probably true parasites. Insect viruses always cause the formation of crystals in the host-cells. Many are entirely intranuclear; others appear to begin development in nuclei, but multiply mainly in the cytoplasm (see Smith, 1955*b*). One large insect virus, apparently containing DNA, has been extracted in crystallizable form (Williams and Smith, 1957).

The phages (Boyd, 1956) infect their bacterial hosts by means of a protein 'tube' through which the DNA component is injected into the interior of the bacterium. Infection in some plant viruses can only occur through an insect vector; and in some vectors the virus multiplies. Such elaborate mechanisms of infection presuppose a biologically long period of adaptive evolution in the viruses concerned, whatever their origin may have been. Infective plant viruses can be obtained in pure crystalline nucleoprotein form, and in one case (Smith, 1956) a virus has been found in the form of minute crystals inside infected plant-cells. Two vertebrate viruses have recently been obtained in crystalline form—the poliomyelitis virus

(Schwerdt and Schaffer, 1955), and the Coxsackie virus (Mattern and du Buy, 1956)—and crystalline arrays have been seen inside cells infected with adenoviruses (Kjellen, Lagermalen, Svedmyr and Thorsson, 1955). Infectivity (in tobacco mosaic and probably in other viruses) depends solely on the RNA, while the protein appears to protect the RNA, for example against ribonuclease (Gierer and Schramm, 1956). The RNA behaves as a single infectious molecule, with molecular weight of about 3×10^6 (Ginoza and Norman, 1957). For recent work on vertebrate viruses, see Haebner (1957). The infective component of encephalitis virus also appears to be RNA: infective RNA can be extracted from ascites tumour cells previously infected with Mengo encephalitis (Colter, Bird and Brown, 1957).

Recent work indicates that the tobacco mosaic virus consists of a central rod of RNA surrounded by a tubular coat of protein. Empty protein tubes sometimes occur, but are non-infective. The two components can be artificially separated and later recombined to form an infective virus particle (Fraenkel-Conrat and Williams, 1955). When RNA from a variant strain producing distinctive symptoms is combined with protein from a standard strain, the resultant reconstituted virus produces the symptoms characteristic of the variant strain (Fraenkel-Conrat, 1956), showing that the genetic properties of the virus reside in its 'germ-plasm' of RNA (see also Gierer, 1957). Recently Commoner *et al.* (1956; and see review by Anon., 1957*a*) have separated the RNA component of standard tobacco mosaic virus into 3 fractions. All of these can be combined with the protein component, and then give three 'new' viruses, differing from each other and from the original virus in the symptoms they produce.

Further, a 'highly artificial' type of RNA has been obtained by polymerization of adenosine and other nucleotide diphosphates with the aid of an enzyme from the nitrogen-fixing bacterium *Azotobacter vinelandii* (Grunberg-Manago, Ortiz and Ochoa, 1956). It does not consist of the usual four types of nucleotide 'arranged in a sequence of genetic significance.' When this aberrant RNA is mixed with protein from standard tobacco mosaic virus, artificial viral particles are formed. These are indistinguishable in visual appearance from normal virus, but are not infective (Hart and Smith, 1956). Finally, Zamenhof *et al.* (cited in *Sci. Amer.*, Feb. 1957, p. 67) have even produced an 'artificial gene'—an abnormal type of DNA by which the bacterium *Escherichia coli* was transformed (p. 85), and then gave rise to various new and peculiar 'mutants.'

2. VIRUS LATENCY: MASKED VIRUSES, PROPHAGE, ETC.

The points of greatest interest for the cancer problem are (1) the 'masking' and 'unmasking' of viruses; (2) the intimacy of their association with host components. As with many tumour viruses, plant viruses may become modified by transfer to another host (Bawden, 1956). A classical case of masked virus is that of King Edward potatoes. No King Edward plants show any symptoms of disease (though they are low yielders), but all will infect other potato strains and other species (tomato, etc.) with the virus disease *paracrinkle*. Transmission can be effected by grafting, sap inoculation, etc. Visible particles in King Edwards were assumed to be the paracrinkle virus; if so, they would be 'masked' or latent. They are serologically related to latent carnation mosaic virus (Kassanis, 1954, who mentions other wholly latent viruses).

It has often been assumed that some normal constituent of the King Edward cells acquires what I may call viral autonomy when introduced into a different environment. That such transformation of a normal host component into an actually or potentially infective virus is possible is suggested by the occasional detection of single plants of various species containing a virus for which, though it can be artificially transmitted, no natural means of transmission exists (Smith, 1952; 1954, p. 82). However, recent work by Kassanis (see Rothamsted, 1956, and Kassanis, 1957) shows that King Edwards do normally contain paracrinkle virus and that this is an aberrant strain of the widely occurring virus S. Its loss of capacity for insect (*Aphis*) transmission is presumably a degenerative character evolved during a long period of propagation by tubers. By growing excised apical meristems from shoots of tubers on nutrient agar, Kassanis obtained virus-free King Edwards.

In some phages there is a clear relation between latency of effect and intimacy of association with host (Lwoff, 1953; Jacob, 1954; and see Glass, 1957; Catcheside, 1957). In certain so-called *lysogenic* strains of host bacteria, the virus exists in a non-lytic state of so-called prophage or provirus, the prophage particles being in some way incorporated with the genetic system of the bacterium. Probably one prophage particle is attached to one locus in each bacterial genome, and replicates at the same rate, though it is possible that the prophage actually replaces some or all of the bacterial gene. Each type of prophage has its own specific locus of attachment. Curiously enough these prophage loci are closely clustered in two regions of the bacterial chromosome: all the prophages attached in one region can be 'induced' (i.e. caused to revert to the active phage state) by ultraviolet irradiation (see below), while those attached in the other region cannot. It appears that most bacterial species show lysogeny—i.e. harbour 'temperate' phages capable of entering the prophage state (see Med. Res. Council, 1957*b*, p. 40).

Burnet (1955, 1957) uses the term *eclipse phase* to denote the period of virus masking, during which replication does not occur and the structure of the virus particle is presumably reorganized to become adapted to the metabolism of the host-cells, and in particular to become linked up with their protein-synthesizing mechanism. See Campbell (1954) for an eclipse phase in an acute erythroleukaemia of chickens and L'Héritier (1955) for a valuable general discussion.

3. BACTERIAL TRANSFORMATION, INFECTIVE INHERITANCE, VIRAL REPRODUCTION

Mention must also be made of the *transformation* of certain bacteria by DNA from other bacterial strains, first discovered by Griffith (1928); (Ephrussi-Taylor, 1951; and see Lederberg, 1952, 1957, and chap. III in McElroy and Glass, 1957). According to Stacey (1956) the DNA appears to be intimately associated with specific capsular polysaccharides. Even particles of purified DNA can in certain conditions be made to enter a living cell, become an integral part of its genome, and act as alleles, apparently replacing a recessive or relatively inactive allele in the nuclear gene-complex.

In *transduction*, a phage acts as a vector transporting genetic material (usually one 'gene' at a time) from one host to another, e.g. in Salmonella (Lederberg, 1955). Hartmann (1957) gives a comprehensive review of this extraordinary process, which in some respects resembles that by which prophage becomes incorporated in or attached to the genetic system of lysogenic bacteria.

DNA from a strain of Diplococcus pneumoniae resistant to *A*.methopterin will transform a susceptible strain so that it acquires considerable resistance. This interesting fact shows that resistance to folic acid analogues like *A*.methopterin is accompanied by an alteration in the strain's DNA components; this may be of importance in regard to the mechanism of resistance in leukaemic cells (Drew, 1957).

Cavalli and Lederberg (1953; and see Hayes, 1952; Catcheside, 1957) have discovered 'infective inheritance,' in *Escherischia coli.* Here the mechanism by which genetic recombination occurs between the chromosome of the female-like acceptor and the chromosome-fragment extruded into the acceptor by the male-like donor, appears to depend on the phage-like particle carried in prophage-like form by the donor and released at conjugation. In some organisms, such as *Pneumococcus* and *Salmonella*, 'infective inheritance seems to have taken over the role of hybridization . . . through fertilization' (see also Haldane, 1955). Such facts will illustrate the possibilities of synergy between extrinsic and intrinsic components of a cellular system.

In all lysogenic strains of bacteria occasional individuals will be lysed; in them the prophage becomes 'unmasked' or detached, and proceeds to multiply disproportionately in the usual way. Environmental agencies can 'induce' active phage from prophage; e.g. ultra-violet irradiation somehow detaches certain prophages from the bacterial genome and frees them for replication.

The reproduction of phages is also relevant. Instead of duplicating itself as do genes, chromosomes and cells, a phage breaks down into a number of sub-units, all of them capable of replication (and of deviating host proteins to their own use). During this period, genetic recombination between two strains of phage can occur. After a certain time the sub-units are reaggregated into fully developed phages capable of lysing their host-cell and infecting new ones (see *Cold Spr. Harb. Symp. quant. Biol.* (1946), **2**, 33; Haldane, 1955; Luria, 1951, 1953; Cairns and Watson, 1956). The analogy between the masking and unmasking of tumour viruses and the alternation of prophage and active phage virus is striking.

Crick and Watson (1956) suggest that all small viruses are made of a number of identical sub-units of two kinds—protein and nucleic acid—and must therefore be either spherical or rod-like. If so, disaggregation of the nucleic acid into sub-units might be a prerequisite for some type of virus latency. The influenza virus is interesting for us because it not merely utilizes host proteins, but incorporates them as they stand (Smith, Belyavin and Sheffield, 1955). The resultant system is thus a compound unit derived from two different sources. *Per contra*, immunological tests do not indicate that normal host components form an integral part of the structure of the Rous sarcoma virus (Rubin, 1957). See also Glass (1957) and Huebner (1957).

4. A NON-PATHOGENIC VIRUS

Carbon dioxide sensitivity in *Drosophila* (L'Héritier, 1948) is due to a transmissible agent with all the properties of a virus, except that it is not pathogenic, and is normally transmitted (to about 20% of the offspring) by the egg cytoplasm, and to a certain extent by the sperm. It can also be artificially transmitted by grafts or haemolymph injections. Like Kappa in *Paramecium* it has a differential growth-rate, multiplying more slowly at high temperatures than normal *Drosophila* proteins, so that flies can be 'cured' of it by high temperatures during periods of active cell-division. Like other viruses, it can 'mutate.' One mutant is thermoresistant (r^+ as against normal r^-), so that flies containing it cannot be 'cured' by exposure to 30° C. Another is normally incapable of infecting the eggs and therefore of hereditary transmission (g^- as against normal g^+). However, when r^+g^- and r^-g^+ strains are simultaneously inoculated, some of the descendants are thermoresistant, as if there had been recombination of g^+ and r^+ from the two strains (Duhamel and Plus, 1956).

L'Héritier (1955) in an important paper finds that some strains rendered sensitive to CO_2 by artificial infection become 'stabilized': the virus combines with host components to become the equivalent of prophage in lysogenic bacteria (see above). He also cites similar examples from other types of virus.

5. PARAMECIUM AND THE CANCER PROBLEM

Sonneborn (1954, and *in litt.*) has drawn attention to the bearing of non-nuclear inheritance in *Paramecium* on the cancer problem (see also Beale, 1954). The Kappa particles which render their 'killer' bearers lethal to non-killers (sensitives) are probably not parasites of rickettsia type, as was once suggested; though reaching up to 1μ or over in length, they apparently consist of pure DNA protein (Sonneborn, *in litt.*). The killer substance, paramecin, which is correlated with their presence, is also composed of nucleic acid (Van Wagtendonk, 1948). They are large paragenes of unknown origin, in a semi-independent relation with the 'host' cells (cf. plant plastids, though these are based on RNA). Under special conditions they can be either infective or contagious of new 'host' cells. They can mutate, but are also under control by 'host' genes. One Kappa mutation inactivates the Kappa particles, robbing them of their capacity to produce killer substance. Such inactive mutants are perhaps comparable with prophages in lysogenic bacteria, latent viruses and 'masked' tumour agents.

The main *Paramecium* gene affecting Kappa is *K*. Double recessives (*kk*) cannot be infected with Kappa. Absolute increase in dosage of the dominant allele *K* permits an increase in the number of Kappa particles in the cell, with a corresponding increase in lethal effect. There is here a possible analogy with genes for tumour-proneness; in virus tumours, certain alleles might permit a higher concentration of virus in the cells. The fact that increase in the number of *K* alleles through polyploidy increases Kappa and its killing properties is significant in relation to the frequency of polyploidy (and aneuploidy) in tumour tissue (pp. 43, 45). Kappa cannot reproduce as fast as *Paramecium* itself; accordingly it is progressively bred out of the system in conditions permitting very fast host fission-rates. Darlington (1948) has suggested that similar differential reproduction-rates of intracellular components during tumorigenesis may lead to the eventual loss of some cytoplasmic replicating units controlling growth and proliferation.

Finally, most mutations in *Paramecium*[1] permit an increase in Kappa number and in lethality. This implies that the growth and multiplication of Kappa have been brought under strict control by the host genetic system, and that any defect in that system is likely to weaken the control. In such a balanced host-paragene system, any mutation deleterious to the host is likely to permit the competing paragenes to multiply more quickly (cf. Crosby (1956), who suggests a similar mechanism to account for pseudo-Lamarckian regulation of growth-rate in unfavourable conditions). This is obviously analogous to the presumed upset of the growth-regulating control system of normal tissues by carcinogens. It also illuminates the at first sight surprising fact that a great diversity of carcinogenic factors can issue in a single type of result, viz. tumorous growth.

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[1] Most mutations studied have been induced by ultra-violet, and are not single-gene mutations, but either euploid or aneuploid.

The presence of Kappa markedly increases the respiration-rate of *Paramecium*, though it reduces the activity of the cytochrome system: possibly the metabolic activities of Kappa compete with those of the mitochondria. The time-relations of the process are interesting. Increased K-dosage produces no change in Kappa concentration for eight generations, and the subsequent increase may take a further six. Again, when two types of Kappa with different reproduction-rates are introduced into one host strain, several cell-generations are needed for the replacement of the slower by the faster type. These facts seem relevant to the well-known time-lag between application of carcinogens and appearance of tumours. Sonneborn (*in litt.*) considers that the natural dimorphism of killer and sensitive found in nature is a true balanced morphism (see Huxley, 1955), Kappa conferring a disadvantage in various conditions of stress (e.g. when oxygen supply is limited), an advantage in others (e.g. higher metabolism in optimum conditions).[1]

The genetic constancy of tumour tissue (like that of all products of normal histo-differentiation) is presumably mediated by differences in the cytoplasm, since the chromosomal genetic system remains essentially similar in all tissues (but see p. 112). The antigenic system of *Paramecium* sheds some light on this problem. There are a number of nuclear genes concerned. Each permits the appearance of a certain array or spectrum of antigenic types of cytoplasm. Within each array, one type may be transformed into another by environmental agencies, notably X-radiation and ultra-violet. In the process, some component of the cytoplasm is altered, and persists in altered form so long as conditions are unchanged. Such non-genic alterations of metabolism, persisting through many cell generations, are akin to the *modulations* of Weiss (see Waddington, 1956). Changes of this type are presumably important in tumorigenesis. But it must be noted that nuclear genes still exert some control—they determine what range of antigenic types is possible, just as cancer-proneness genes determine what types and incidence of tumours are possible.

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[1] Sonneborn also mentions the complication that Kappa may apparently be parasitized by a virus, which may sometimes persist in strains which have lost all Kappa particles!

6. ORIGIN AND BIOLOGICAL RELEVANCE OF VIRUSES

The facts concerning virus tumours thus confront us with basic biological problems of infection, symbiosis and differentiation. Altenburg (1946*a*, *b*, 1948) suggests that 'ultra-microscopic organisms akin to viruses' are universally present as useful symbionts in the cells of cellular organisms. These *viroids*, as he calls them, he regards as representatives of the precellular or 'naked gene' stage of life, which have survived through adopting a symbiotic habit. Pathogenic viruses would then be derived from viroids during evolution (though some of them are doubtless ancient). The mutual infection of cellular organisms and viroids took place in the remote Archezoic past; but viruses would have to develop infectivity if they were to become permanently established (cf. the fact that non-infective plant viruses seem occasionally to arise *de novo*; p. 84).

He also suggests that the antigenic types of *Paramecium* depend on autocatalysts in the cytoplasm; these would then be similar to the self-reproducing cytoplasmic substances that are often postulated as determining the characters of differentiated tissues in a higher animal. However, change from one antigenic type to another is under environmental control and is reversible. The character of a tissue, on the other hand, is in most cases irreversibly determined, though the determination may be non-genetic (epigenetic), as with the effect of vitamin A on epithelial differentiation (p. 95).

Darlington (1944, 1944 *bis*, ch. 6, 1948, and Darlington and Mather, 1948, p. 211 f.), on the other hand, regards viruses (apart from some of the larger kinds) as autogenous—that is, originating from normal cytoplasmic proteins (nucleoproteins) which have become abnormal; and also autonomous in being able to infect fresh cells. In respect of tumours, the usual series of events would then be as follows. (*a*) Initiation of production of abnormal autocatalytic (paragenic) protein—virtually a plasmagene, except that it is not transmitted through the egg (presumably because it arises in a somatic tissue). (*b*) Acquisition of potential but not actual autonomy; the paragene can be transmitted by cell-free filtrates, but is not normally infective (e.g. Rous sarcoma). Darlington calls this stage a provirus. (*c*) Acquisition of normal infective capacity (e.g. milk agent in mice). Other authors have formulated the same general idea—that viruses are derived from normal paragenic cell units—in somewhat different terms (see Luria, 1953, ch. 18). Dr. Darlington tells me that inbreeding of many insects will cause the appearance of viruses. If so, this would presumably be the result of genetic imbalance causing a normal cytoplasmic component to acquire the abnormal properties and the partial autonomy of a virus.

Actually we need a further category—that of the active virus which becomes latent or masked, as with Shope papilloma in domestic rabbits or prophage in lysogenic bacteria. This would be a secondary provirus stage. In many cases the step from latent to patent virus is reversible, as in phages and in masked viruses.

Finally, there is the theory, based on analogy with pathogenic bacteria, that viruses were originally free-living organisms which have all become parasites, but parasites of a peculiarly intimate kind, not merely utilizing host products, but *metabolic parasites*, utilizing some of the host's metabolic processes to their own advantage, or even incorporating some of its proteins. There are two variants of this. (1) The original form was a free-living sub-microscopic subcellular organism of 'naked gene' type. (2) The original form was of bacterial type; during its parasitic evolution its organization was progressively (or suddenly) degraded, perhaps by some process akin to lysis, but leaving some nucleoprotein component as a virus, to form part of a replicating metabolic system.

We may never be able to decide with certainty between these three possibilities; the weight of evidence, however, is against origin of viruses from independent organisms which have become parasitic, save for the rickettsia types. Altenburg's viroid theory is of great interest for broad evolutionary biology, but in relation to cancer is very similar to the host-protein theory. If viroids are universal symbionts, then they are normal constituents of all higher organisms.

It is, however, hard to see how the theory could apply to viruses based on RNA, which could not well have been the main nucleic acid component of a 'naked-gene' organism.

There are some recent facts which favour, or at least make plausible, the theory of virus origin from normal host constituents. Weiss (1955) has adduced evidence that all growing tissues liberate substances, presumably of protein nature, which have a specific effect on the growth of other tissue of the same type, by acting as 'anti-templates' (perhaps steric complements) to the specific growth templates (catalysts) of the tissue. And Medawar and his associates (Billingham, Brent and Medawar, 1956) have shown that healthy grafts, and presumably host tissues too, are constantly liberating into the circulation antigenic substances, which unpublished results indicate are particles of DNA nucleoprotein.

In his *Principles of Animal Virology*, Burnet (1955, and see Burnet, 1957) concludes that the findings concerning cancer viruses 'are in accord with what would be expected if they had an immediate or relatively recent endogenous origin,' and that this presumption would be strengthened if it could be proved that an 'eclipse phase' (masking) occurs when they enter susceptible cells (see p. 74 f.).

Pirie (1956) suggests that viruses (and all viroid paragenes) are best regarded as components of a compound metabolic system; to employ a metaphor, they have stolen a ride on the host-cell's metabolism, and are steering it in abnormal directions. One might call such a system *intergenetic*, as implying that it involves the interaction of two distinct types of replicating component. Though the virus would not survive unless some of the products of the system were fresh virus, the replication may often be inaccurate, resulting in non-infective dummy viruses, which Pirie calls 'companion proteins' (see also Jeener, Lemoine and Lavand'homme, 1954).

Whatever the evolutionary origin of viruses, the way in which they can integrate host proteins into their own structure, and their capacity for becoming latent and more or less completely incorporated into the host's genetic or metabolic system, are of great general interest, while the work on *Paramecium* shows how paragenes may become semi-independent, and how they may provide a basis for morphic intraspecific differentiation.

Surveys such as that of Lederberg (1952, 1957) and the symposium edited by McElroy and Glass (1957) make it clear that a new field, that of cellular genetics, is being opened up. We are beginning to discover the nature and extent of the non-nuclear mechanisms by which strains of cells, whether somatically differentiated or free-living, and symbiotic systems involving cells and sub-cellular units, transmit their distinctive characters. In this field the concept of the paragene (or plasmid, or whatever other term is used to denote non-nuclear replicating units based on DNA or RNA) may come to play as important a role as that of the gene in mendelian genetics. And to this the study of tumours will have made an essential contribution.

III

TUMOURS AND EPIGENETICS

1. INTRODUCTORY:
EPIGENETICS AS THE ANALYTIC STUDY OF DEVELOPMENT

I AM adopting Waddington's (1956) useful term *epigenetics* to denote the analytic study of individual development (ontogeny) with its central problem of differentiation.

The method by which tissues and organs differentiate in the course of normal development is at the moment the main blank space in biology's map. Waddington (1956) gives a valuable account of the present state of our knowledge and of our ignorance. Certain concepts, such as induction, evocation, competence, individuation and morphogenetic field, have introduced a preliminary degree of order into the subject, but we know little of the precise steps taken by epigenetic processes, of the biochemical factors involved, and above all, of what determines the replicable specificity of differentiated tissues.

2. DIFFERENTIATION, METAPLASIA, AND METABOLIC DEVIATION

Students of cancer cannot therefore yet expect much help from general epigenetics. However, amphibian metamorphosis illustrates the differential reactivity ('competence') of different tissues in different species to the same substance (hormone), resulting in the excessive growth of some tissues, and the destruction and resorption of others.

Some agencies result in abnormal differentiation. Thus Ganter (1956) and Ganter, May and Kourilsky (1956) have shown in mice that intra-ocular grafts of lung tissue, especially from young or foetal animals, frequently induce 'an anarchical proliferative reaction' in the host's corneal epithelium, and also in skin epithelium engrafted simultaneously with the lung tissue, resulting in the production of tubular formations and cornified spheres and cysts. Contact is necessary for the reaction, and no other tissues so far tested are effective.

Then we have the important work of Fell and her associates (Fell and Mellanby, 1953; Fell, Mellanby and Pelc, 1954; Fell, 1956, 1957) and of Weiss and James (1955), showing how differentiation may be switched into a radically new channel; the addition of vitamin A to cultures of presumptive or even differentiated embryonic chick epidermis causes its irreversible differentiation into a mucus-secreting, often ciliated epithelium, with a quite different sulphur metabolism. In differentiated epidermis, the undifferentiated basal layer gives rise to the new 'metaplastic' type of tissue; if then deprived of vitamin A, it would presumably revert to the production of normal epidermis.

Similarly, Wilde (1955) found that phenylalanine, which is necessary for neural crest differentiation, will switch undifferentiated ventral ectoderm into tissue of neural crest type; and it has been known for a long time that the lens (in urodeles) produces some substance which 'holds the retina in check': when the lens is removed, retinal cells at the margin of the retina immediately start multiplying (presumably after dedifferentiation) to form the quite different tissue of a new lens (see Waddington, 1956, p. 306).

In the lamellibranch *Pecten*, grafts of ripe ovary into the adductor muscle eventually degenerate inside a cyst formed by host-organ fibroblasts; and these then turn into columnar and eventually ciliated epithelium (Drew, 1911; and see Huxley and de Beer, 1934, p. 213). Here again we have a case of induced metaplasia in a stroma (p. 96).

Such tissue-transformations are often included under the head of *metaplasia*. This term is also applied in cancer research to the apparent development of carcinomas into sarcomas, to the development of malignancy in the fibroblastic (host) stroma of carcinomas, to the development of new tissue types in tumours derived from a particular tissue, and also to reversible changes in appearance both of malignant and of pathological non-malignant epithelia.

This last phenomenon is clearly similar to that discovered by Fell and Mellanby; indeed, the formation of squamous cell tissue in glandular tumours and the converse formation of mucous epithelium from skin cancers are well known. The frequent 'metaplastic' development of tissues like bone and cartilage in tumours of mesenchymal origin is doubtless also comparable, and appears to depend on the pluripotency of mesenchyme tissue (Willis, 1953), just as the interconvertibility of epidermis and mucous epithelium depends on the pluripotency of the undifferentiated basal cells.

The term 'metaplasia' is also applied to the easily reversible switches of cell-form to be seen in cultures of the same tissue in different conditions, which, however, do not involve any loss of the original determination of the tissue (see Waddington, 1956, p. 14).

The transformation of carcinomatous into sarcomatous tissue is probably apparent only (Willis, 1953), being due to mutational change to higher proliferation and therefore to greater structural dedifferentiation (p. 97).

Metaplasia is thus a purely descriptive term, which covers modulations (normally reversible), irreversible switch differentiations of various degrees of magnitude, and irreversible changes due to mutation. Switch transformations of tissue-type of large extent seem only to occur through undifferentiated cells (basal layer in the Fell phenomenon) or dedifferentiated cells (blastema in regeneration, p. 99).

Metaplasia in tumours may throw light on general epigenetics. Thus the above-mentioned development, in the host stroma of some carcinomas, of malignancy (and of a growth-rate matching that of the carcinoma) and the very existence of true mixed and composite tumours (see Willis, 1953), appears to be due to an induction by the carcinoma. Certainly fragments of mammary tumours in the anterior eye-chamber stimulate the growth of fragments of some (but not all) normal new-born tissues in the same site (Browning, 1952). Further analysis of such growth-induction by a tissue (or region) of high growth-rate could be of general importance for the study of normal development.

Tumours do not constitute a specific type of differentiated tissue like muscle or epidermis. Tumour development (tumorigenesis) is thus not an abnormal type of differentiation: indeed it is not differentiation in any proper sense of the term (see Ruben, 1955). It results from a deviation or abnormal type of metabolic activity which may occur in any type of differentiated tissue.[1] This is supported by two sets of facts. (1) In general, tumours retain the inherent specificity (though not all the visible specific characters) of the particular tissues from which they arise. This applies to undifferentiated as well as to differentiated tissues; teratomas form many kinds of tissue and organ because the cells they arise from are undifferentiated and therefore pluripotent (see, for example, Fekete and Ferrigno, 1952; Berrill, 1943). (2) As will be seen later, tumorigenesis often and perhaps always proceeds by a series of mutation-like steps, occurring as individual chance events in isolated (though sometimes multiple) foci, followed by differential multiplication of cell-types, instead of by a continuous (statistical) process of transformation affecting all cells simultaneously and essentially uniformly, as in normal tissue differentiation. The series of steps may differ in rate and result in two sub-lines of one tissue undergoing tumorigenesis, though in identical conditions (p. 106).

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[1] Graff (1956) calls cancer a 'cellular aberration.'

3. DEDIFFERENTIATION

The undifferentiated *appearance* of tumour cells is due not to a reversal of determination—that is, of the processes which determine tissue specificity—but to the disappearance of some or all of the visible histological structures exhibited by the normal differentiated tissue. This is doubtless in large part a result of the well-known general fact that rapid replication is antagonistic to the development or maintenance of full histological structure: thus, the basal layer of the epidermis is fully determined as epidermal tissue; however, the characteristic keratinization cannot develop in it, but only in the non-multiplying cells which it produces. In addition, the deviation of tumour metabolism from respiratory to glycolytic (p. 107) is stated by Warburg (1956*a*, *b*) to render the cells less capable of maintaining structural differentiation.

Dedifferentiation is used for three quite distinct types of process whose only common feature is the loss of visible histological structure: (1) reversal of determination and return to a more pluripotent state, as in the regeneration blastema of urodele limbs (Waddington, 1956, p. 356; Ruben, 1956). Waddington calls this 'true dedifferentiation'; (2) loss of visible structure alone, owing to rapid replication, as with most tissues in tissue culture; (3) reduction of general vital activities and entry into a resistant resting state, as in encysted Protozoa. An adequate terminology is urgently needed to distinguish these different processes (see Ruben, 1956*b*). Until more suitable terms are agreed, I shall use *dedetermination* or pluripotent dedifferentiation for the first type; *hypoplasia* or hypoplastic dedifferentiation for the second;[1] and *anabiosis* or anabiotic dedifferentiation for the third.

The dedifferentiation of active tumour cells is thus hypoplastic, but does not indicate return to an embryonic or pluripotent state, as is often misleadingly supposed. However, exposure to certain unfavourable conditions, for instance, deep-freezing and anoxia, especially in combination, will bring about anabiotic dedifferentiation in some cells of some tumours (Craigie, 1954)[2]. Dehydrating agencies will reinforce the process. The resultant cells are spherical, highly dehydrated, inactive and extremely resistant. Under suitable treatment they will return to an active state as tumorous cells of the original type. This phenomenon is important for general biology, as the first occasion on which specifically differentiated vertebrate tissues have been shown to be capable of anabiosis. It will be of great interest to see whether any normal (non-tumorous) tissues have the same capacity. It is also important for the cancer problem. The recurrence of an operated cancer after a long period (e.g. of malignant melanomas after 10–20 years), as evidenced by metastases in other sites, may depend on the survival of such anabiotically dedifferentiated cells in unfavourable sites (e.g. without adequate blood-supply) and their redifferentiation when conditions permit (Hadfield, 1954).[3]

4. TUMORIGENIC DEVIATIONS OF METABOLISM

Many deviations of metabolism are known, leading to various pathological or lethal states. That underlying tumorigenesis is characterized by leading to autonomy instead of to normal organization and differentiation. Tumorigenesis therefore does not involve one or a few canalized *epigenetic pathways*, to use Waddington's useful phrase, which natural selection has equipped with self-regulating mechanisms during evolutionary time. It follows any tracks which happen to lead to autonomy; and such tracks will only become available if the normal growth-regulating mechanism has been broken down.

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[1] *Anaplasia* would be more suitable, but has unfortunately been pre-empted by pathologists to mean total loss of visible structure, and (usually but probably incorrectly) to imply reversion to an 'embryonic' (pluripotent) state.

[2] Low temperatures also alter their antigenic properties (p. 113).

[3] Dr. A. Haddow tells me that recent work indicates that freezing may alter the inherent properties of cancer strains, notably in reducing their specificity.

The work of Witschi on the effects of delayed fertilization in Anura (1952; and see Huxley and de Beer, 1934, p. 96) illustrates the role of metabolic deviation in tumorigenesis. Moderate delay switches the metabolism of the eggs towards maleness, eventually producing 100% male tadpoles. Further delay leads to various morphogenetic abnormalities, while extreme delay results in tumorous outgrowths in the late blastula, followed by early death. According to Briggs and Berrill (1941) the growth-disharmony evidenced by the outgrowths results not from abnormally high proliferation by the tumorous areas, but from the depressed proliferation-rate of the remaining areas. If this is confirmed, we should have a unique form of tumorigenesis by differential reduction of growth.

Tumours are characterized by lack of organization as well as by non-limited proliferation. Failure of normal tissues to develop organization has been shown by Spemann (1942), Bosaeus (1926), Andres (1950), Waechter (1951) and Dürken (1926); see also Holtfreter (1944). Following Belogolowy (1918) they grafted fertilized amphibian eggs, or fragments, or tissues of early embryos, into the body-cavity, etc., of older organisms. Many of them developed into unorganized masses of different tissues, and some appeared to be tumorous or even malignant. Other workers, however, dispute these results.

Faukhauser and Stonesifer (1956) found that decapsulated urodele embryos implanted under the skin of adults (not in their body-cavity) developed into typical teratomata, characterized by unorganized growth, but with presence of atypical parts of various organ-systems, and by transformation of the epidermis into closed vesicles within the mass of the growth, but they were never invasive. Waddington (1956, p. 307; 1940, p. 123) found that early blastemas of regenerating urodele limbs grafted into the adult body-cavity formed unorganized masses of undifferentiated cells, with some resemblance to malignant tumours (see also Sheremetieva-Brunst, Brunst and Figge, 1952). Repetition of such experiments in varied conditions would be of value.

Needham (1942, p. 139; and see Berrill, 1943) suggested that tumours acquired autonomy by escaping from the local individuation field. However, when this hypothesis was tested by grafting a tumour on to a urodele limb and then amputating the limb through the region of the graft, the intense individuation field of the regenerating limb had no effect on the tumour (Ruben, 1955, 1956*a* and *b*, who failed to confirm Rose and Wallingford, 1948). Waddington (1935) had previously suggested that individuation fields exert a certain growth-controlling function, and that animals or organs capable of regeneration might therefore be less susceptible to tumours; this should be investigated.

In Urodela supernumerary limbs and tails have been induced by some chemical carcinogens (Breedis, 1952), by irradiation (Brunst, Sheremetieva-Brunst, Barnett and Figge, 1950), by ligation and by various tissue extracts, notably cartilage (Fedotov, 1949), and by grafts (Ruben, 1955). Limb regeneration in late stages of Anura, where it normally does not occur, has been elicited by wounding, especially in combination with strong sodium chloride solutions (Rose, 1944; Polezhayev, 1946; and see Waddington, 1956, p. 305). For any regeneration, pluripotent dedifferentiation followed by blastema-formation is necessary. Polezhayev believes that high histo-differentiation (as in late anuran limbs) stands in the way of dedifferentiation; this is the converse of rapid proliferation leading to histological dedifferentiation in tumorigenesis and elsewhere.

Interaction between nervous system and tumours has already been referred to in invertebrates (p. 29). In vertebrates (male guinea-pigs) Coujard and his colleagues (1954, 1955, 1956) claim to have produced (*a*) benign salivary gland tumours by damaging the general sympathetic system; and (*b*) one malignant osteochondrosarcoma by lesions of the diencephalon. A detailed account of the procedures and results would be of value.

More recently Coujard and Heitz (1957) have obtained a high proportion (over 50%) of osteochondromas in the hind feet of guinea-pigs by injecting a section of the sciatic nerve with weak phenol solutions. This treatment apparently only damages the sympathetic fibres, the others being protected by their medullation.

By their high fixation of Gallium 67, some of the tumours should be classed as malignant, though no metastases had been formed at the time of publication. The tumorigenic effect of direct damage to sympathetic nerve-fibres is greater and more rapid than that of damage to the diencephalon or to sympathetic ganglia (see above). Coujard and Chevreau (1957) also record various adenomatous tumours induced by sympathetic damage, some of which became malignant.

Petrov (1956) refers to work by V. K. Fedorov at Leningrad and by R. V. Bulasashvili at Tbilisi. The former concludes that 'the tone of the cortex of the brain' influences the responsiveness of mice to carcinogenic influences. The latter states that the first visible effects of the application of chemical carcinogens to the skin of mice were histological changes in nerve-fibres and ganglion cells, the implication being that these caused the later appearance of tumours.

Hamburger (1954) and Levi-Montalcini, Meyer and Hamburger (1954) have established the converse effect, of tumour on nervous system. Fragments of mouse sarcomas (but not carcinomas) engrafted on to chick embryos or their chorio-allantoic membranes, or in tissue-culture, exert a powerful effect on sympathetic and spinal ganglia, causing precocious differentiation and much increased growth (up to 600%) of the ganglia. A large excess of nerve-fibres grow out, and in embryos flood neighbouring organs. No effect is produced on fragments of spinal cord or heart. The agent is a diffusible non-dialysable heat-sensitive substance, apparently a nucleic-acid-free protein (Cohen and Levi-Montalcini, 1957). Snake venom exerts a similar but much more potent effect (Cohen and Levi-Montalcini, 1956; Levi-Montalcini and Cohen, 1956).

5. NATURAL AND EXPERIMENTAL CARCINOGENESIS

Carcinogenesis in higher vertebrates, notably experimental carcinogenesis, has been exhaustively reviewed (see Haddow, 1949, 1952, 1953, 1955; Hartwell, 1951; Boyland, 1952; Greenstein, 1954, ch. 3; H. L. Stewart, 1955; Hieger, 1955; Berenblum, 1956; Cook, 1957). Here I can only mention a few of the more important points. In the first place, effective carcinogenic agencies are immensely various, including genetic imbalance, single genes, parasites (Fibiger's nematode cancer in rats,[1] Bilharzia cancer in man), various chemical substances, including such a common tissue constituent as cholesterol (Hieger and Orr, 1954; Hieger, 1957), various types of radiation, certain prolonged irritations, etc. This is to be expected. Any agency capable of pushing cell-metabolism in a certain general direction —that of upsetting the normal growth-balance so as to favour non-limited replication—is capable of initiating a tumour. However, different agencies may differ in activity, in resultant cytological changes (Koller and Casarini, 1952), and in the types and sites of the tumours they induce.

Cook (1957) mentions that a given carcinogen may induce tumours more quickly in one species, less so in another. He also points out that, as one might expect, the painting of most carcinogenic hydrocarbons on the skin induces carcinomas, while their subcutaneous injection induces sarcomas. However, 9, 10-dimethyl-1, 2-benzanthracene is an extremely potent carcinogen for mouse and rabbit skin, but wholly non-carcinogenic for rabbit subcutaneous tissue (Berenblum, 1956). Isotopes of elements selectively taken up by particular organs will naturally produce tumours in the organs concerned, e.g. radio-iodine in the thyroid (Bustad, Marks, George, and Seigneur, 1957) or radio-strontium in bones.

A possible new tumorigenic factor has recently been discovered by Menkin (1956*a*, *b*, *c*). He finds that a diffusible growth-promoting substance from inflammatory exudates is responsible for reparative proliferation. Repeated injections of this into non-pregnant rabbit mammary glands produce persistent tumour-like ('pre-cancerous') growths, sometimes with squamous metaplasia.

A parallelism between carcinogenic and mutagenic capacity has often been postulated and frequently occurs (see, for example, Bird and Fahmy, 1953; Auerbach, 1955; Fahmy and Fahmy, 1956). With physical carcinogens (radiation) it is fairly close, but with chemical carcinogens may be less close and is sometimes absent (see Auerbach, 1949). Burdette (1952) obtained a moderate increase of tumours (but not of lethal mutations) of a *Drosophila* strain with a 20-methylcholanthrene aerosol.

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[1] Various authorities now consider that the growths described by Fibiger were not truly neoplastic: see Willis, 1953, p. 56. Another possible nematode-induced tumour is that of the canine oesophagus described by Seibold *et al.* (1955) as being associated with the nematode parasite *Spirocera lupi*. See also pp. 25, 27.

It appears that embryonic tissues are more responsive to chemical carcinogens than adult tissues. Embryonic tissue treated with methylcholanthrene and grafted into adults develops tumours much sooner than do adults similarly treated (references in Shimkin, 1950).

(*a*) *Tumorigenesis as a multi-step process*

Berenblum (1954) has made the important discovery that in some kinds at least of experimental chemical carcinogenesis in mouse skin (and probably elsewhere) two steps—initiation and promotion—are involved. Initiation is sudden, irreversible and is envisaged by Berenblum as similar to a somatic mutation, either in a nuclear gene or a cytoplasmic paragene. Promotion, on the other hand, demands a considerable time (latency). The suggestion is that a lengthy process of differential multiplication or excess production of some substance is needed before the threshold is crossed which permits non-limited growth and the appearance of any visible tumour (benign papilloma in the case of the skin).[1] Platt (1955) has elaborated this two-stage theory of carcinogenesis by suggesting that initiation involves a mutation in a cell leading to the differential multiplication of its descendants. The development of malignancy occurs later, and involves one or more further steps (see below).[2] A single application of the chemical carcinogen used as initiating agent will not alone result in tumours, but induces a condition in which tumours will appear *either* through further applications of the carcinogen *or* through repeated applications of a so-called co-carcinogen like the irritant croton oil, which by itself will hardly ever produce tumours (see also Foulds, 1954; Glücksmann, 1945).[3]

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[1] Hieger (1955) gives a beautiful series of microphotographs illustrating the development of an experimental tumour in a mouse.

[2] Occasionally, experimentally induced mouse tumours are malignant *ab initio*. Here, presumably, the step to malignancy has been taken during the promotional stage.

[3] Boutwell, Bosch and Rusch (1957) have recently established that croton oil is definitely carcinogenic to mice, but only to certain strains. They conclude that it acts synergistically with sub-effective doses of stronger carcinogens, in the same way as do two different carcinogenic hydrocarbons applied sequentially, each in sub-effective dose.

Poel (1956) finds that surprisingly low concentrations of chemical carcinogens may induce tumours; for example, painting with 0·01% solutions, or administration of 1 μg. tri-weekly for 40 weeks. As one would expect, with genetically mixed mouse populations, there is a very wide range of variation in response (see p. 62 f., and Pybus, 1955). With irradiation the latent period is generally longer than with chemical carcinogens (see, for example, Glücksmann, 1951). Here, too, co-carcinogenic factors may operate; thus irritation by means of silicon particles much reduces the latent period. Brues (1954) reviews the general subject of carcinogenesis induced by ionizing radiations.

Tumours induced by tarring often regress if tarring is stopped; if it is renewed, they recur at the same sites. The process may be repeated two or three times (Foulds, 1954). Regression is very rare in the processes of normal tissue-differentiation.

Once autonomy has been attained, the original carcinogen is no longer required for the continued growth and further evolution of the tumour (p. 65). In this respect, chemically-induced animal tumours are broadly similar to crown-gall tumours in plants (p. 29 f.). Nothing is known as to whether the genetic determination of tumours involves chemical carcinogens as an intermediate step in tumorigenesis.

Nordling (1953), following Muller's (1951) theoretical deductions, claims, on the basis of statistical analysis, that six or seven distinct steps must be taken before malignancy is attained. Intensity of malignancy, sometimes involving the capacity to grow in foreign species, may be later increased in a further series of steps (see also Armitage and Doll, 1954).

(*b*) *Acquisition of malignancy*

Armitage and Doll (1957) recall that, over a wide age-range, mortality from most types of human cancer rises in proportion to a high power (about the 6th) of the age. Their mathematical analysis of the mortality data now indicates that this fact need not demand a corresponding number of mutational steps, as previously envisaged (Armitage and Doll, 1954), but can also fit a two-stage theory, in which the first step is due to a mutation causing increased proliferation (see above), and the second step is not the result of a continuous process of ageing, as envisaged by Platt (1955), but of a second mutational event.

Ambrose, Abercrombie, Heaysman, James and Lowick (see Abercrombie and Heaysman, 1953; Ambrose, James and Lowick, 1956) have made the important discovery that malignancy does not necessarily involve increased proliferation, but a change in surface properties such as electrical charge, malignant cells in tissue-culture being therefore more mobile and no longer tending to adhere. This accounts for the invasive properties of malignant tumours (see also Dale, 1954). The lower calcium content of malignant cells (De Long, Coman and Zeidman, 1950) may also play a role, since calcium is known to be necessary for the adhesion of cells in many organisms (see Huxley and de Beer, 1943, p. 401). For the importance of adhesiveness in normal vertebrate development see Holtfreter (1943), Waddington (1956, p. 444f.); and for its experimental modifiability in sponges see Spiegel (1955).

Berenblum suggests that during promotion there is an inhibition of differentiation (in mouse skin, of the basal layer), which allows the cell-colony produced by division of the 'initiated' cell to reach a required critical size. On this view, chemical carcinogens could have both initiatory and promotional effects, while co-carcinogens are only promotional. This is supported by Rous and Rogers (1952), who found that constant regenerative proliferation in repeatedly tarred areas largely inhibited tumour-formation, and wholly prevented malignancy. The apparently paradoxical fact that carcinogenic agencies in general inhibit growth would also help to account for the long latent period of the resultant tumour. Indeed Haddow nearly twenty years ago (1938 *bis*) found that in some cases the administration of chemical carcinogens might bring about the regression of established (autonomous) tumours.

Haddow (1949) suggests that nitrogen mustards (and possibly other carcinogens) produce both these effects by direct combination (cross-linking) with the nucleo-protein fibres of the chromosomes. The same author (1953) inclines to the view that Berenblum's promotional stage involves the progressive elimination of key proteins concerned in normal growth-regulation. A similar view is expressed by Cook (1957), based on the fact that the azo dyes which induce liver tumours become attached to liver proteins. Other workers suggest a somewhat similar theory, of enzyme-deletion (see Miller and Miller, 1952). Kotin, Falk *et al.* (1956) find that tumour induction by methylcholanthrene and dibenzanthracene is markedly reduced or abolished by their partially reduced derivatives (cf. the action of nucleic acid analogues, p. 111, and of 'anti-vitamins'). Alexander (1954) reviews the reactions of various carcinogens with host macro-molecules, and Badger (1954) the relation between chemical constitution and carcinogenic activity.

Rusch (1956) stresses the competition for nutrients (substrates) between metabolic mechanisms concerned with all special cell-functions as against that of replication; this results in an incompatibility between differentiation and proliferation. On this view carcinogenesis depends on the abnormal (disorderly) abolition, total or partial, of one or other of the special cell-functions, with a corresponding deletion of a specific protein. The deletion of a special cell-function not only interferes with cellular differentiation but also makes more nutrients available to the replicating mechanism. Successive deletions may be necessary for the attainment of autonomy and a high degree of malignancy. The concept of alternative developmental pathways involving interdependent and competing metabolic mechanisms is firmly based on studies on simple organisms (see e.g. Beadle (1951) and Ephrussi (1953)). Its extension to cover deviations of metabolism (p. 96) could clearly be fruitful for the cancer problem.

The fact that a series of steps is required, and that many at least of the individual steps appear to resemble mutations in being chance events and not the result of a statistical process, is shown by Sanford, Likely and Earle (1954) (see also Burk, in Warburg, 1956; and Southern, Moore and Rhoads, 1957). From a single normal fibroblast in tissue-culture, two strains were grown, both of which eventually developed malignancy without application of carcinogens: but one developed it much earlier and with much greater intensity than the other. Earle (1944), Firor and Gey (1945), Gey, Gey, Firor and Self (1949), and Goldblatt and Cameron (1953) had previously shown that untreated (and treated) tissue-cultures of normal rodent tissue could sometimes become malignant, and both Leighton, Kline and Orr (1956) and Leslie, Fulton and Sinclair (1956) have shown it with human tissue; see also Berman, Stulberg and Ruddle (1957), Coriell (1957), A. E. Moore (1957). We must suppose that the abnormal conditions of tissue-culture can favour tumorigenic processes. They certainly favour deviations of differentiation (Parker, 1957). The culture of fibroblasts in media permitting only their slow growth may lead to some sub-lines 'mutating' to macrophage type with higher proliferation-rate (Parker, 1932, 1933). As reported at the Tissue Culture Association's decennial conference in 1956 (Anon., 1957*b*), many animal cell-strains derived from single cells now exist, some on protein-free media. They may exhibit marked morphological and probably physiological divergence from their prototypes, and often show hypoplastic dedifferentiation (p. 98), tending 'to lose some of their specific characters, or at least to put them in abeyance,' remodelling themselves in relation to the new conditions.

(*c*) *Warburg's theory; anaerobiosis*

Warburg (1956*a* and *b*) also believes that a series of steps are involved, each leading to a new replicable state, but maintains that the two main processes concerned are: (1) irreversible damage to the respiratory mechanism of the cell, followed by (2) a compensatory increase in glycolytic metabolism, both aerobic and anaerobic (see Adams and Quastel, 1956).[1] His point is that once the energy requirements of the cell fail to be met owing to (1), only such cells will survive as mutate (or modulate) towards (2). The damage to the respiratory mechanism may be exerted either by non-specific respiratory poisons like urethane, or by specific ones like arsenious acid. The damage is usually though not necessarily effected in a series of small steps. Such a mechanism would account for the long latent period in carcinogenesis, but not for the sudden 'mutational' initiation postulated by Berenblum.

Burk and Schade (1956) have devised a simple manometric test for cancer tissue, confirming its high glycolytic activity (fermentation: respiration ratio). However, Leslie, Fulton and Sinclair (1956) have tested the glycolytic activity of various cell-strains—three from human carcinomas, one from liver of a 20-week human embryo, which showed various characteristics of malignancy, and three from human embryonic skin, kidney and lung tissue respectively. They also refer to the results of other workers on the glycolytic activity, in aerobic conditions, of myelocytic and lymphocytic leukaemia cells, and of normal leucocytes. Contrary to Warburg's views, all the malignant and presumably malignant cells showed markedly lower rates of glucose-consumption and lactic acid production than normal leucocytes and embryonic cells. Insulin increased the metabolic quotients of malignant cells, but decreased those of normal cells. It will be interesting to extend these results to cell-strains from other types of tumours, malignant and benign.

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[1] Intermittent anaerobiosis may induce malignancy in tissue-culture (Goldblatt and Cameron, 1953).

Graff (1956) makes the point that cancer cells are 'so sluggish aerobically' that they have to parasitize the host and draw on it for substances needed in the metabolic cycle. He states that tumours are unable to metabolize either acetate or pyruvate. In tissue-culture the tumour-cells' deficiency in oxidative mechanisms restricts their growth to the surface, whereas many normal tissues can grow throughout the culture medium. Tumour cells appear to be unable to 'acclimatize' to low oxygen-tension. At any rate, in height-acclimatized animals brought down to low levels, engrafted with tumours and returned to high altitudes, the tumours were markedly inhibited in their growth and often showed total regression.

There are probably elements of truth in all these views. But whatever the upshot of further research, two things seem clear. First, that tumorigenesis does not result from an abnormal differentiation, but from a particular deviation of metabolism; and secondly, that malignancy is not due merely or even primarily to very high proliferation-rate, but to a qualitative change in surface properties.

While some of the steps in tumorigenesis thus undoubtedly seem to be mutational, in the general sense of involving sudden irreversible changes in individual replicating elements (chromosomes, genes or paragenes), there is some indication that in chemical carcinogenesis simultaneous multiple 'mutation' in a number of cells (foci) may also occur. Further, some stages in tumorigenesis appear not to be sudden but progressive, involving the differential multiplication or elimination of cells or cytoplasmic components (Berenblum's promotional stage, perhaps Warburg's respiratory damage, enzyme-deletion, etc.).

6. ENDOCRINE-DEPENDENT (CONDITIONED) TUMOURS

The important subject of endocrine-dependent tumours has been reviewed by numerous authors, e.g. Burrows and Horning (1952), Furth (1953), Hieger (1955, p. 44), Oberling (1952, p. 102 f.), Greenstein (1954, ch. 4, 6), Bielschowsky, 1955, and Shimkin, 1956. Such tumours are caused by an excess of some normal endocrine activity, and may regress when it ceases. Sometimes a cyclic proliferation in a non-endocrine tissue under endocrine control is over-stimulated by excess production of the relevant hormones; for instance, in mammary cancer owing to excessive oestrone production by the ovary. In other cases an endocrine feed-back mechanism is upset and leads to over-activity of, and tumour-formation in, an endocrine tissue; for example, the pituitary's gonadotropic hormone stimulates the ovary to secrete oestrone, which in turn inhibits the secretion of the gonadotropic hormone by the pituitary. If the oestrone is prevented from reaching the pituitary, an excess of gonadotropic hormone is secreted, causing continuous over-stimulation and eventually cancer of the ovary. Oestrogens will also regularly cause lymphatic leukaemia in mice and other animals (though rarely in man) (Stanley, 1957).

The tumorigenic effect of continuous over-stimulation of activity by hormones is clearly closely similar to that caused in plants by continuous over-stimulation of growth by excess auxin (p. 31).

As an example of the co-operation of genetic and endocrine factors, in CE mice ovariectomy always produces carcinoma of the adrenal cortex, in DBA mice nodular hyperplasia only, in C57 Blacks hardly any effect; the changes could be prevented by hormone replacement.

The complexity of the interrelations between different members of the endocrine system is shown by the following facts: (1) thyroid atrophy caused by radioactive iodine results in excess secretory activity by the pituitary and the growth of tumours in it; (2) thiouracil depresses the thyroid's secretory activity; this brings about excess secretion of thyroid-stimulating hormones by the pituitary, which then causes thyroid tumours (see Furth, 1953, Bielschowsky, 1955; also Rosin and Rachmilewitz, 1954). Grafts of these latter tumours will take in thiouracil-treated mice, but not in normals. Greenwood (*in litt.* and 1956) has found that fowls kept in uniform 'optimum' conditions show a progressive decline in egg-production in successive years from above to below normal, followed eventually by an abnormally high incidence of malignant tumours, mainly of the ovary and oviduct. This appears to be correlated with the fact that in uniform conditions no winter cessation of egg-laying occurs, so that ovarian activity is continuous. As Shimkin (1956) points out, the key position of the pituitary in the endocrine hierarchy is reflected in its importance relative to endocrine-dependent tumours; since 'the gonadotrophic, adrenocorticotrophic and thyrotropic hormones of the pituitary stimulate the appearance of neoplasms in the respective target organs.'

Such tumours, whose persistence depends on endocrine or other conditions in the body, Furth (1953) calls *conditioned* (as against *autonomous*, or as I would prefer to say, *non-conditioned*), e.g. the renal tumours induced by stilboestrol in hamsters (Horning, 1956 *bis*); the uterine and abdominal fibromas produced by small doses of oestrogen, but regressing when its administration is stopped (well analysed by Lipschütz, Iglesias, Bruzzone, Fuenzalida and Riesco, 1948; see also Gelfant, Meyer and Ris, 1955); and the malignant melanomas which arise (or occur) during human pregnancy (presumably as result of excess melanocyte-stimulating hormone from the pituitary), but may regress permanently after parturition (Allen, 1955; Pegum, 1955); see also Lasnitzki, 1955 (prostate), and Horning, 1956 (kidney). Endocrine-dependent tumours may therefore sometimes be successfully treated, at least temporarily, by endocrine therapy. Furth (1953; see Kandutsch, Murphy and Dreisbach, 1956) finds that conditioned tumours, e.g. of the pituitary caused by abolishing thyroid function, show negligible dedifferentiation, either histological or physiological. This is because the conditions for non-limited growth are provided by the host. As soon as such tumours become non-conditioned (i.e. capable of growth in normal hosts) they show dedifferentiation.[1]

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[1] Horning (1957) finds that chemically-induced prostrate tumours in mice were endocrine-dependent while they remained glandular in type, but independent when, after serial transplantation, they underwent squamous metaplasia.

7. CRITICAL POINTS IN THE TUMORIGENIC PROCESS

Biologically, and I would add medically, one of the most important facts about the cancer process (tumorigenesis) is that it is divisible into two distinct main phases, the latent and the patent. During the former, which includes Berenblum's initiation and latent period, the induction of the tumour has been set in train, but there are no clinical manifestations: during the latter the tumour is not only manifest, but has become autonomous. Two critical points are involved—that of irreversible initiation and that of (normally) irreversible autonomy. After the second we can treat cancer, but cannot prevent it; before the former we may prevent cancer, but cannot (as yet) apply any treatment.[1]

Before the second critical point is reached, there sometimes and perhaps always intervenes a third phase, of manifestation without irreversible autonomy. This is the conditioned phase, just noted. Besides endocrine-conditioned tumours it includes, for example, skin tumours which appear during the promotional stage of chemical carcinogenesis but regress when carcinogen application is stopped (p. 104).

8. METHODS OF TREATMENT

The chief methods of treatment, apart from operation, are (1) irradiation; and (2) chemotherapy (see reviews by Stock (1954), Tatarjet (1956) and Skipper, Heidelberger and Welch (1957), and symposium on anti-tumour agents, *Proc. roy. Soc. Med.* 1956, in the press), most interestingly by administering antimetabolites such as 'fake' components (analogues) of DNA such as 8-azoguanine and other purine and pyrimidine analogues (Brown, 1954*a*, *b*; Hayhoe, 1955; Graff, 1956; Greenstein, 1954, ch. 7), notably fluorinated pyrimidines (Heidelberger *et al.*, 1957), antagonists such as aminopterin (see Gelfant *et al.*, 1955; Skipper, 1953), and enzymes such as ribonuclease and desoxyribonuclease, but also by cytostatic agents. Holman (1957), on the basis of a hypothesis of tumorigenesis involving a catalase-hydrogen peroxide mechanism, caused complete regression of adenocarcinomas in 72 rats, and improvement of 4 inoperable human tumours, by administration of H_2O_2 (in dilute solution in drinking water). The fact (cited in Skipper, Heidelberger and Welch, 1957) that some malignant cell-strains have a high capacity for incorporating their amino acids not as such but as proteins, may open the door to differential chemotherapy. According to L. F. Larionov in Moscow (cited in Anon., 1956*c*) a new derivative called sarcolysine is effective in cases of seminoma. Belkin and Hardy (1957) found that doses of reserpine and chlorpromazine capable of inducing 'deep tranquillization' of mice, markedly inhibited the growth of a sarcoma, both in solid and ascites form. Further experiments will be needed to decide whether this is a specific effect, or a result of the inanition, dehydration and lowered temperature of the hosts.

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[1] Berenblum (1956) makes what amounts to the same distinction, but restricts the term *carcinogenesis* to the first phase, of induction of the tumour (initiation plus promotion), as against the second phase of its 'growth and evolution.'

All methods depend on the differential sensitivity of tumorous as against neighbouring normal tissue, owing to its higher proliferation-rate, though, as stressed by Greenstein (1954, ch. 7) and Latarjet (1956), different types of cancer are sensitive to different agencies and chemotherapeutic substances.[1] There is thus always a risk of damaging the host, and the more effective the treatment is in damaging the tumour, the greater the risk to the host. Since tissues are more likely to show specific differences in their (cytoplasmic) RNA than in their (nuclear) DNA, forms of chemotherapy specifically aimed at preventing RNA replication might be more efficacious for tumours.[2] However, King and Briggs (1955) have shown that the nuclei, and so presumably the DNA, of cells of amphibian embryos do undergo irreversible differentiation during gastrulation. Henstell and Freedman (1952) have found the interesting fact that the concentration of desoxyribonuclease varies inversely with the maturity (differentiation) of tissues, being highest in leukaemia blast cells. Sellei, Eckhardt, Hartai and Dumbovitch (1956) find that a new nitrogen mustard compound (linked with mannitol) 'strongly inhibits the growth of metastasizing tumours and proliferating leucocytes,' especially in chronic lymphoid leukaemia. 2-chloroethyl methonesulphonate effectively inhibits both solid and ascites Walker rat carcinoma (Haddow and Ross, 1956). Serological treatment has also been attempted (see Hauschka, 1952), but here the differential between a tumorous tissue and its parent host-tissue is in most cases not likely to be sufficiently great. However, if any characters of malignant tissue, such as its distinctive surface properties (p. 105), prove to be associated with antigenic differences, a fruitful line of approach would be available. L. A. Zilber in Moscow (cited in Anon., 1956*c*) claims to have found specific antigens in tumour-cells (not present in non-tumour cells): one seems to be a specific protein component of the tumour-cell, present in the mitochondrial and microsome fractions. See also Korngold (1957).

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[1] Waddington, Feldman and Perry (1955) find similar differential sensitivity to purine analogues in different organs of vertebrate embryos; the specific sensitivities of different organs also differ as between chick and newt. Inhibition by analogues has already been successfully applied to plant virus diseases (Matthews, 1954, 1955) and by ribonuclease administration to influenza virus replication (Le Clerc, 1956).

[2] The affinity of lithium salts for RNA (Stacey, 1955) and their well-known inhibiting effect on early differentiation (see Huxley and de Beer, 1934) suggest that they might be efficacious against some tumours.

9. METABOLIC BALANCE, TISSUE RESORPTION AND TUMOUR REGRESSION

A biologist is inclined to feel that more attention should be given to the relations involving metabolic equilibrium and growth-partition which must exist between a cancer and the parent (host) organism, since both draw on a common nutritional substrate, and share a common vascular supply (see, for example, Le Page, Potter, Busch, Heidelberger and Hurlbert, 1952. This is confirmed by the fact that high tumour-incidence (in mice) can usually be reduced by a restricted diet. Mammary cancer can be virtually abolished by this means (Tannenbaum, 1945). Thus the occasional 'spontaneous' regression of a cancer must depend on some reversal of the tumour's 'physiological dominance.'[1] When carcinoma 755 and sarcoma 180 are present in the same mouse, the growth of the former is inhibited and of the latter enhanced, presumably owing to unequal success in competing for some factor needed for growth (Antopol, Graff, Zagal and Agate, 1952).

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[1] For cases of reversal of physiological dominance in normal equilibrium-systems see Huxley and de Beer (1934, pp. 292f., 425) and for growth-partition Huxley and de Beer (1934, p. 421) and Huxley (1932).

The regression and resorption of denervated, reversed, ultra-violet-irradiated and xenoplastic urodele limbs, normal and regenerating (Butler, 1951; Butler and Blum, 1955; Schotté and Butler, 1944; Durante, 1955), and the necessity of innervation for the dedifferentiative phase leading to blastema formation in their regeneration, may well have a bearing on tumour-resorption. So may the facts concerning the resorption of almost all *Drosophila* and of some anuran tumours (p. 77) during normal metamorphosis, the resorption of urodele limbs grafted on to anuran larvae only *after* the host's metamorphosis (Durante, 1955), and the success of Helff (1955) in causing tumour-resorption by extracts of amphibian tissues undergoing regression during metamorphosis. The autolysis of tissues leading to perforation of mouth, anus and gill-slits in normal development at sites where ectoderm and endoderm come into contact (see Waddington, 1956, pp. 287, 294) may also be relevant; as also the regression of rabbit uterine tissues when in contact with the degenerating tissues of dead embryos in process of resorption (Henderson, 1954). Some histo-compatibility genes (see p. 61) cause complete regression of tumours which have started to grow after engrafting (Borges and Kvedar, 1952). Regression of tumour grafts can be either enhanced or inhibited by injection of suitable tissue antisera or lyophilized tissues (Snell, 1952*b*; Kaliss and Molomut, 1952). More recently Kaliss (1957) has made it probable that homografts of normal or tumorous tissue into mice have two distinct immunological effects on subsequently engrafted tumours: (*a*) an early toxic effect; (*b*) a later enhancing effect, permitting the survival and growth of the tumour. Enhancement can also be obtained with passive antisera. There was great variability in the vigour and growth of the surviving tumours, which was only partly dependent on major histo-compatibility genes. Kaliss was unable to obtain enhancement of growth of grafts of normal tissues, but others have had success in rats and partial success in rabbits. Tumours as such do not appear to possess specific immunogenetic properties (though the greater ease of enhancement of tumours, as against normal tissues, may be correlated with their characteristic growth-properties). No enhancement of leukaemias has been obtained. Franks, McGregor, Shaw and Skublics (1956) find that the marked enhancing effect of spleen homogenates on the growth of a mouse tumour was much reduced with homogenates of spleen from animals with induced immunity to the tumour (by X-ray cure, etc.). Bielschowsky (1955) gives numerous examples of the regression of endocrine-dependent cancers induced by appropriate hormone treatment or endocrine adjustment—by castration and hypophysectomy (in mammary cancer), by oestrogens (in mammary and prostatic cancer), by progesterone (in cancer of the cervix), etc.

In general, treatment should be focused on the patient-tumour system, not merely directed against the tumour in imaginary isolation. The apparent success of thyroid or thyroxin treatment in reducing the risk of recurrence of operated female breast and genital cancer (Loeser, 1954), if confirmed, might well be due to a tilting of the metabolic balance in favour of the host as against the growth of latent or potential tumour-cells. It is in any case important to note that hyperthyroidy is associated with a low female cancer-incidence, hypothyroidy with a high one (Loeser, op. cit.).

Southam, Moore and Rhoads (1957) inoculated or grafted cancer tissue into normal individuals and into advanced incurable cancer patients with short life-expectancy. In the normal men, the initial inflammatory reaction was more marked and lasting, and the number of positive takes much less (6/15 as against 21/23). The difference was not due to a failure of immunological capacity in the cancer patients, since they produced antibodies towards viruses. Further research is needed to decide whether it was a result of their general debility, or was related to the fact that they were already suffering from cancer.

10. METHODS OF PREVENTION

This last fact provides a bridge to the subject of prevention. Wherever a causal relation or a significant correlation is established between an environmental condition and a type of tumour, some degree of cancer-prevention becomes possible. Thus in endemic goitre areas cancer-incidence will be reduced by iodine or other appropriate treatments. Bather and Franks (1952) find that thyroxin antagonizes (and thiouracil enhances) the action of chemical carcinogens (though it is without effect on established tumours). If so, keeping thyroxin at optimal level might well reduce general cancer-incidence (cf. p. 155).

All occupational, habitudinal and environmental cancers can be prevented, or more properly speaking their incidence drastically reduced, by appropriate measures.[1] Thus khangri will be prevented when Kashmiris no longer warm themselves by suspending charcoal warmers inside their clothes (Oberling, 1952, p. 19), and chutta when Asians cease smoking cigars with the lighted end inside the mouth (H. L. Stewart, 1955). Bilharzia cancer of the bladder (Oberling, 1952, p. 65), could be prevented by antiparasite measures, and malignant *erythema ab igne* (Peterkin, 1955) by changed domestic habits. Improved dental care coupled with a decline in pipe-smoking has brought about a steady fall in the incidence of cancer of the mouth (Smithers, 1956). Pybus (1955) points out that the incidence of chimney-sweep cancer was much lower where greater personal cleanliness was habitual. He considers that cancers are often 'dirt diseases,' and that 'only the regular washing of our faces saves most of us, living in a smoky atmosphere, from epithelioma.' Certainly the use of soap and water can prevent many cancers dependent on soot, tar-impregnated fumes, etc.

The most striking example of habitudinal tumorigenesis is that of lung cancer correlated with heavy smoking, especially of cigarettes (Doll, 1955; Hill and Doll, 1956; Doll and Hill, 1956; Stocks and Campbell, 1953; Hieger 1955, ch. 4; see p. 63). Between 1940 and 1954 the annual mortality from lung cancer in England and Wales increased over threefold, and is now about 17,000, some three times that from road accidents, while that from other major cancers remained almost constant (see graph in Joules, 1956*a*). Joules (1956*b*, summarized in *Nature*, **178**, 1152) states that 11·9 million British males of sixteen and over smoke an average of 15·3 cigarettes a day. The Medical Research Council (1957*a* and *b*) states that in Britain, male heavy cigarette-smokers have a death-rate from lung cancer nearly 40 times that of non-smokers,[1] probably 1 in 8 being destined to die of this disease. The death-rate from lung cancer in Britain has risen steadily for 35 years or so, and is still rising. In the 10 years before 1955 it more than doubled, and is now responsible for over 5% of male deaths. They also point out that the risk of death from lung cancer goes up roughly in proportion to the number of cigarettes smoked per day,[2] and is greater in those who continue to smoke than in those who give up the practice. They estimate that the latent period needed for the manifestation of lung cancer caused by smoking is between 25 and 50 years.

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[1] For occupational cancers see Hunter (1955), Goldblatt and Goldblatt (1956); for environmental cancers, Hueper (1952), H. L. Stewart (1955) and pp. 37, 63; for geographical variation of cancers, Internat. Soc. Geogr. Path. (1955); Hieger (1955, ch. 2); Dorn and Cutler (1954); Dorn (1955); for habitudinal cancers, pp. 38, 60.

The heterogeneity of cancers is well brought out by the fact that different histologically distinguishable types of lung tumour show different degrees of correlation with smoking. Further, several occupations (e.g. chromate working) predispose to lung cancer of various types, but the total incidence of occupational lung cancer is very small.

Wynder and Bross (1957) adduce statistics to show that oral (and lip) cancer is strongly correlated with the smoking of pipes and moderately with that of cigars; in females it is also correlated with citrus deficiency. Heavy intake of alcohol increases the risk of oral cancer many times, probably because of associated nutritional deficiencies. Lip cancer is very rare in U.S. negroes. They cite Sanghvi *et al.* (1955) to show that in India tobacco smoking alone is especially correlated with oro-pharyngeal and tonsillar cancer, betel-chewing alone with the cancer of lip and anterior buccal cavity, and a combination of the two with a (considerably higher) incidence of cancer of the hypopharyngeal cavity and the base of the tongue. A great deal of research is now being done on the subject, and we can prophesy that within a few years the chemical carcinogens responsible will have been identified.

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[1] Wynder (1957) estimates that the chances of an Englishman smoking 40 or more cigarettes a day developing lung cancer is 70 times that of a non-smoker: the corresponding estimate made by an American research committee (*The Times*, March 25, 1957) is 27·5 times.

[2] Edwards (1957) maintains that the damage to the lungs per cigarette per day decreases slightly with the number of cigarettes, so that there would be a higher incidence of lung cancer (and, he claims, of tuberculosis) if everyone smoked 15 cigarettes a day than if half the population smoked 30 a day and half were non-smokers. For bronchitis, however, the damage per cigarette per day *increases* with the number smoked.

The only other types of neoplasia which have shown a comparable rate of increase of incidence in recent years are the leukaemias (Witts, 1957). These show a much lower incidence in Japan than in the U.S.A., and in U.S. negroes than in U.S. whites. In general, incidence increases with higher standard of living, but the precise environmental agency responsible for the increase is unknown.

The British Empire Cancer Campaign (1957) in its 34th Annual Report, provides confirmatory evidence of the causative association between smoking and lung cancer. Five hydrocarbons, including the well-known carcinogen benzpyrene, have been found in tobacco and also in cigarette smoke: they appear to be produced during the process of drying the tobacco leaves. Lung tumours have been produced in a quarter of the few rats whose lungs were inoculated with tobacco tar extracted from the filters of cigarettes smoked by human subjects. The U.S. Surgeon General, on the basis of an investigation by a study group of the U.S. Public Health Service (reported in *The Times*, July 13, 1957), states that 'laboratory and biological data provide contributory evidence that excessive smoking is one of the causative factors in lung cancer.' It is intellectually pitiful to find that such judicial and cautious statements, which are based on evidence carefully accumulated over many years, and merely assert such clearly established facts as that excessive cigarette smoking markedly increases the probability of contracting lung cancer, should be dismissed by spokesmen for the tobacco industry as only 'the opinion of some statisticians,' etc. (*The Times*, loc. cit.). Every credit, however, should go to the tobacco industry for launching large-scale research on the subject.[1] This is clearly the channel through which the problem may eventually be solved, and men and women may be able to enjoy the pleasures and benefits of smoking without running the risk of a premature and painful death for themselves and much grief and sorrow to their families.

Thus the incidence of lung cancer will inevitably fall if people stop heavy cigarette smoking. It will also fall if the carcinogenetic substances involved are prevented from reaching the lungs (see Marshall, 1954) or removed from cigarettes. Some of these at least appear to be higher aromatic hydrocarbons formed by pyrolysis owing to the high temperatures in lighted cigarettes. Since their major precursors are natural hydrocarbons occurring in the waxes covering the tobacco leaf, removal of these substances, e.g. by elution with hexane, should markedly reduce the carcinogenic effects of smoking. These can also be reduced by filters, which still permit satisfactory taste.[1]

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[1] Among a number of interesting results mentioned in the Report of the Medical Research Council (1957*b*), I may mention the finding that hyperplasia of human foetal lung in tissue-culture can be induced by 3,4-benzpyrene.

The incidence of lung cancer in comparable classes of smokers and non-smokers is higher in urban than in rural areas (Stocks and Campbell, 1955), so that it could also be reduced if the agent or agencies causing higher urban incidence—whether coal smoke, petrol or diesel-engine exhausts (see Thomas, Evans and Hughes, 1956, who obtained tumorous growths in mushrooms and toadstools by exposure to tar and diesel oil fumes and diesel exhausts; p. 33) or anything else—is identified and appropriate steps taken.[2]

The conclusion to be drawn from the evidence is definite: increased smoking increases the probability of developing lung cancer. Unfortunately, the significance of such a statement is not clear to many people. Obsessed by the naïve idea of finding a single cause for every effect, they shake their heads and say that the evidence is only statistical, as if that invalidated it. In point of fact, every scientific law is statistical, and all that physicists can tell us about the electrons within the atom is the probability of their occupying any particular position. Further, the degree of probability, whether of an electron occupying a particular position or of an urban English cigarette-smoker developing lung cancer, can be quantitatively determined, often with high accuracy. Such quantitative determinations of probability in respect of lung cancer at once point the way for research into the material causes at work. And once these have been discovered—as they undoubtedly will be within a few years—steps can be taken to nullify them. Meanwhile, the statistical discoveries of the last few years show definitely that it is possible for people to reduce the probability of developing lung cancer by cutting down their smoking. They have the quantitative data which will permit them to reach a reasonable compromise and strike a balance between the undoubted advantages of moderate smoking, at least to some people, and the equally undoubted risks of lung cancer, in spite of the great individual variations in both.

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[1] Goodhart (1956) suggests that the increased incidence of lung cancer correlated with heavy cigarette smoking may be partly due to smoking determining the *site* of the cancer, rather than the fact of its occurrence, the increase in lung cancer being then partly compensated for by decrease of cancer in other sites, while Hill and Doll (1956) reject this view. The suggestion appears improbable on general principles, but could be checked one way or the other by appropriate statistical surveys.

[2] N. Petrov in Leningrad (cited in Anon., 1956*c*) claims to have isolated considerable amounts of 3,4-benzpyrene from the smoggy air of Leningrad, and considers that this, and not cigarette smoking, is the chief cause of the increased incidence of lung cancer.

11. METHODS OF RESEARCH

Geographical and ethnic surveys of various types of cancer may give the possibility of reducing incidence, for example, in goitre areas or in regions where betel chewing is common (Oberling, 1952, p. 19), and by providing clues as to carcinogenic habits and practices. Peruvian Indians have a very low incidence of cancer (Cambridge Andean Expedition Report, 1957). Confirmation of the findings of Prof. Reyniers of the Lobund Institute, Notre Dame University (*in litt.* 1956), that the incidence and virulence of cancer is markedly reduced in totally sterile (germ-free) animals, might open the door to numerous hopeful possibilities. In *Drosophila*, however, Mittner showed that rearing in aseptic conditions had no effect on tumour-incidence.

Finally, a proper follow-up of the known facts concerning the difference in incidence of various types of cancer between man and lower animals might be important. The most striking difference is the much higher incidence in man of cancers of the alimentary tract. This presumably depends on the 'unnatural' and often deficient diet of our species, as exemplified by the probable carcinogenicity of overcooked fat (Oberling, 1952, pp. 112, 117),[1] or by liver cancer correlated with dietary deficiency (see Hieger, 1955, p. 24). Cook (1957) states that the lung tumours induced in animals by chemical carcinogens are usually adenomas, never bronchocarcinomas which are the common type in man. Further investigation might well indicate various means for reducing the incidence of many such cancers. As Hieger writes (1955, p. 30), in the field of applied cancer research 'the most important work today is being carried out by a masterly combination of statistics, pathology and demography.'

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[1] Brown and Tappel (1957) have shown that various carcinogens, including 3, 4 benzpyrene and methylcholanthrene, affect fat-oxidation systems catalysed by haematin pro-oxidatively. In conjunction with free-radical lipids produced by haematin catalysis, they appear to give rise to other, stable free-radical carcinogenic compounds.

Meanwhile in pure research Schultz (1956) in a valuable paper points out the value of convergent studies from many fields, and the need to determine all the elements (biochemical unit-processes) involved in the epigenetic pathways (many of them alternative) leading both to normal and tumorous development. With regard to the fact that many agencies and substances, of very different type and constitution, are carcinogenic, he reminds us of an analogy in the field of fertilization and artificial parthenogenesis. Here also workers were puzzled by the wide range of agencies capable of initiating cleavage of the ovum. To-day, as the result of work carried out half a century ago on these extrinsic agents, some of the intrinsic chemical substances important in the process of normal fertilization are being identified. We may hope for similar discoveries as regards the tumorigenic process.

In any case, in the future organization of cancer research it seems clear that comparative studies, cytogenetics, epigenetics, allometric and other growth studies, virology and human ecology should always be included, in addition to intensive biochemical analysis and to the established histological, physiological, pathological, pharmacological, clinical and therapeutic disciplines. If the study of cancer is regarded as an important branch of general biology, both biology and medicine will profit.

C. Summary

1. I employ *cancer* in a broad sense to cover all products of *malignant neoplasia*—carcinomas, sarcomas, leukaemias and ascites tumours—and *tumour* to cover all autonomous neoplasms.

2. Cancer is not a biological entity, but an assemblage of many distinct diseases (malignant tumours) which share the following symptoms: (*a*) replication of abnormal tissue-type, but often with change towards greater malignancy; (*b*) non-limited proliferation; (*c*) some degree of histological and physiological dedifferentiation; (*d*) partial or often complete lack of organization; (*e*) invasiveness, often accompanied by metastasis.

3. *Neoplasia* also includes the development of *benign tumours*, which exhibit symptoms (*a*)–(*d*) above, and have therefore acquired autonomy.

4. Neoplasia does not involve an abnormal form of tissue-differentiation, but a characteristic deviation of metabolism which may affect any type of tissue. Tumours retain the specific determination of the tissues from which they arise, though in hypoplastic manifestation. Tumorigenic metabolic deviation appears to proceed by a series of individual chance (mutational) events, instead of by a continuous (statistical) process affecting all cells simultaneously as in normal differentiation.

5. All autonomous neoplasms can be regarded as the equivalents of new biological species.

6. Though the tumours of lower vertebrates, invertebrates and plants often differ in various ways from those of mammals and birds, there is no reason for regarding them as irrelevant to the human cancer problem. They are autonomous and often malignant neoplasms, and have sometimes been experimentally induced.

7. Neoplastic tumours, whether spontaneous (in nature) (S), experimentally induced (E), or genetically induced (G), have been recorded in the following groups of organisms:

Plants:
- Dicotyledons (S, E, G),
- Basidiomycete Fungi (E).

Animals:

Chordata. Mammalia (S, E, G), Aves (S, G), Reptilia (S), Amphibia (S, E), Pisces (S, G), Urochorda (S, probable).

Arthropoda. Insecta: Diptera (S, G), Coleoptera (S, E), Hymenoptera (S), Lepidoptera (G), Orthoptera (E), Arachnida (S), Crustacea (S, G).

Mollusca. Gastropoda (S), Lamellibranchiata (S), Cephalopoda (E?).

Annelida. Polychaeta (S).

Sipunculoidea (S).

8. The 'cancer spectrum' may differ markedly in different groups, species and breeds.

9. High cancer-proneness exists in some animal species (e.g. dogs, mice, budgerigars, domestic fowls, poecilid and cyprinodont fish) and breeds (retrievers, grey horses, some domesticated goldfish), and low cancer-proneness in others (guinea-pigs, rabbits, pigs, primates; Pekinese, chows).

10. Thyroid tumours develop frequently in salmonids and some other fish in water of low oxygen-content. Their invasiveness is usually not an effect of malignancy, but of the absence of a capsule in the fish thyroid.

11. Attention is drawn to the favourable material for cancer research provided by regions of high localized growth-rate (horns, horn-cores, antlers, etc.), growth-gradients (crustacean appendages and abdomina, molluscan mantle-edges, etc.), endocrine-dependent growth and regression (fowl-combs, larval and metamorphosing Amphibia, stick insects, etc.), nerve-dependent differentiation (Orthoptera), regenerating limbs (Amphibia, Crustacea, stick insects, etc.), late-fertilized Amphibian eggs, graft-tolerant sites (cheek pouches of hamster), crown-gall tumours and non-tumorous plant galls, and toadstools.

12. The rate of development of spontaneous and induced tumours is related to size of species. Further investigation is needed to determine (*a*) if this holds also for size of breed, (*b*) if it is related to rate of development to maturity, or to length of life.

13. Crown-gall tumours in plants are initiated by a bacterium, *Agrobacterium tumefaciens*, which induces an irreversible alteration in the plant's tissue leading to high heteroauxin production and

unorganized tumorous growth. Plant tissues cultivated for long periods on media of high auxin content acquire many properties of crown-gall tumour tissue. Desoxyribonucleic acid extracts from *A. tumefaciens* can transform other species of bacteria so that they induce tumours.

14. Virus tumours in plants are induced by a combination of a specific virus and traumatic injury.

15. Some tumours are determined genetically, with high or complete penetrance, either as a result of gross genetic imbalance in species-crosses (*Nicotiana*, *Platypoecilus*, *Pygaera*), or of chromosomal imbalance (extra *B*-chromosomes in *Sorghum* pollen-grain tumours) or of single genes or systems of genes (*Drosophila* larval melanotic tumours, melanomas in grey horses, human xeroderma pigmentosum, tumour incidence in monozygotic twins, some mammary cancers and leukaemias in mice).

16. The incidence (penetrance) of genetic tumours may vary markedly with diet and other environmental conditions (notably in *Drosophila*), with stage of development (all *Drosophila* tumours regress at metamorphosis), and with genetic background (notably in different inbred mouse strains, which show marked differences of proneness to various tumours). Some single genes affect cancer-proneness, e.g. *yellow* in mice increases the incidence of lung cancer, the *A* blood-group allele in man that of cancer of the pyloric antrum, the *O* allele that of cancer of the body of the stomach.

17. Specific, subspecific and breed differences in cancer spectrum or particular cancer-proneness must have a genetic basis; human 'racial' differences may do so, but may be determined by environment or habits.

18. The incidence of some mammary cancers in mice depends on a combination of genetic factors with a virus transmitted in the milk. Incidence is mediated through the timing of the endocrine-dependent mammary cycle, high incidence occurring in strains where local areas of proliferation persist after the rest of the gland is in regression.

19. Histo-compatibility genes determine susceptibility to tumour transplantation, but have no relation to genetic cancer-proneness.

20. The study of ascites tumours has revealed a wide genetic variance in the cell-populations of tumours, due to polyploidy, aneuploidy, structural changes (notably translocations) and gene-

mutations. The tumour is propagated mainly through a 'germplasm' of modal 'stem-cells,' the remaining more extreme variants constituting its non-transmitted 'soma.' In changed conditions, selection may operate to alter the profile (idiogram) of the variance, with the establishment of a new mode and set of stem-cells (e.g. near-tetraploid in place of near-diploid). Major physiological adaptation of the entire cell-population is not operative. Immunoselection results in new aneuploid modes, with loss of antigenic properties. In other conditions, selection of other mutants may result in higher proliferation-rate or invasiveness. Inbred mouse strains may change their general or particular cancer-proneness, as a result of deliberate or unconscious selection. Thus, though the abnormal ploidies and structural changes often seen in tumours are certainly not the primary cause of tumorigenesis but secondary effects of it, they may provide the basis for further genetic evolution of the tumour.

21. The apparent lack of any marked 'heredity' of cancer in man and wild animal species is due to (*a*) the multiplicity of types of cancer, and (*b*) the genetic heterogeneity of natural populations. Whenever inbreeding is practised, breeds or strains with particular degrees of genetic proneness to particular cancers will be established. The high variance of human populations in genetic cancer-proneness accounts for the fact that very high incidence of known occupational, environmental or habitudinal cancer will only occur with very prolonged and very intense exposure to the carcinogen. Lesser degrees of exposure will result only in a moderate incidence, which yet may be causally significant, as with the increase of lung cancer due to cigarette smoking.

22. Evolutionary selection may affect the manifestation and incidence of cancers in various ways. It may operate (*a*) to produce a relative immunity to tumorigenesis in tissues especially subject to environmental insults, e.g. the nasal mucosa; (*b*) to defer the onset of reasonably common cancers, such as most carcinomas, to post-reproductive ages; this will not be possible with rare cancers such as most sarcomas, or with cancers with very early onset, such as the leukaemias of childhood, or xeroderma pigmentosum. In this latter case selection has produced an alternative mode of palliation, by imposing recessiveness on the deleterious effects of the gene responsible.

Cancers are also subject to a general orthoselective pressure in favour of higher proliferation-rate and greater invasiveness.

23. Viruses, in the sense of filterable, replicable and transmissible (infective) particles in the cytoplasm, containing nucleoprotein, cause a number of tumours, such as Rous sarcoma in fowls, avian leukosis, some mammary cancers and some leukaemias in mice, Shope papilloma in rabbits, and Lucké adenocarcinoma in frogs.

24. Virus tumours cannot be understood without considering the nature and properties of viruses in general. These include insect viruses, vertebrate viruses, plant viruses, bacterial viruses (bacteriophages), rickettsias and non-pathogenic viruses (carbon dioxide sensitivity agent in *Drosophila*).

25. The replicating properties of viruses depend on nucleic acids. Plant viruses are based on ribonucleic acid (RNA), perhaps because they multiply only in non-dividing cells. Most others are based solely or mainly on desoxyribonucleic acid (DNA), though the main nucleic acid component of influenza virus is RNA. Insect viruses always cause crystal-formation in the host-cells, and many are intranuclear, while others begin development in nuclei but multiply mainly in the cytoplasm. Rickettsias are intermediate between other viruses and bacteria and are probably true parasites. Other viruses form part of a compound metabolic system involving host components, one of whose properties is the synthesis of more virus. The 'composition' of viruses, notably as regards proteins, may thus vary with the host's physiological state and genetic properties and with the stage of the virus cycle (e.g. plant viruses can be obtained outside the plant as pure crystalline nucleoprotein, their infective properties then depending solely on their RNA; influenza virus in one phase incorporates host proteins).

26. Viruses are one type of the larger category of nucleoprotein-containing replicating units other than nuclear genes, e.g. plasmagenes, plant plastids and Kappa particles in *Paramecium*. Lederberg calls all these *plasmids*: I suggest the term *paragene*. All appear to be capable of mutation.

27. Some viruses are transmitted through the egg (carbon dioxide sensitivity in *Drosophila*, some mouse leukaemias), others through the mother's milk and (to a lesser extent) the father's sperm (Bittner milk agent in mice).

28. Many viruses in certain conditions become masked or latent, no longer producing host symptoms. Some plant viruses remain wholly masked in certain host-strains (e.g. paracrinkle in King Edward potatoes). In phages latency is achieved through the virus (prophage) becoming attached to a specific locus in the bacterial gene-complex. Some tumour viruses (e.g. Shope papilloma) become masked (non-extractable and non-infective) in rapidly proliferating and highly malignant host-tissues, presumably owing to closer association with host-components; others (e.g. Rous sarcoma) become masked in tumours grafted into old hosts. Some viruses (e.g. adenoidal-pharyngeal-conjunctivitis) become 'unmasked' by repeated passage on solid media. Viruses may produce quite different symptoms in old and very young hosts, and in new (alien) hosts.

29. Active phages and probably other viruses do not replicate by duplication, but by breaking down into numerous sub-units, all capable of replication (and of deviating host proteins to their own use). Recombination occurs between sub-units of different strains. The replication of latent phages (prophages) is synchronized with that of the host genome. This behaviour of prophages has points of resemblance to the genetic 'transformation' of bacteria by means of pure DNA, units of which apparently replace normal recessive alleles in the 'host's' gene-complex; and also to the 'infective inheritance' (sometimes at least mediated by a heavily masked virus-like agent) found in, for example, *Pneumococcus* and *Salmonella.*

30. In *Paramecium* the inheritance of 'killer' properties through Kappa particles capable of replication and mutation (but under partial nuclear genetic control), and the cytoplasmic modulation of antigenic properties within a gene-determined range of antigenic spectrum, are discussed in their relevance to problems of tumorigenesis. One important point is that almost all host gene-mutations permit an increase in Kappa number (and lethal properties), presumably by weakening the host's control over Kappa multiplication. Another is that increase of the number of host *K* alleles by polyploidy (*K* is a dominant allele which is necessary for infection by Kappa) also increases the number and the lethality of the Kappa particles in the cell.

31. With most tumour viruses (*a*) infection is much easier in embryos or very young animals, notably of other species, and then

often causes haemorrhagic or other non-neoplastic disease instead of tumours: (*b*) adaptation to a different host species, subspecies or strain usually alters the virus in various ways, notably its specific infectivity, and the site and type of tumour which it induces. It is possible that inoculation of tumour cells or filtrates into newborn or embryonic hosts will reveal the presence of (masked) viruses in many tumours now considered non-viral.

32. There are various theories concerning the origin of viruses. (*a*) Altenburg's theory. This regards viruses as derived from 'viroids,' supposed to be present in all organisms, and to be representatives of the precellular ('naked gene') stage of evolution, which have survived through adopting a symbiotic habit. (*b*) The autogenous theory, of which Darlington is the protagonist. This regards viruses as originating from normal cytoplasmic nucleoproteins which have become abnormal, autonomous, and eventually infective. (*c*) The parasite theory. This regards viruses as having originally been independent organisms which have become 'metabolic parasites' of a peculiarly intimate kind, utilizing or incorporating some of the host's metabolic processes or products. Some supporters of this theory consider that viruses were always of precellular type, others that they are degraded bacteria.

Theory (*c*) appears to be true for rickettsias; (*a*) is highly speculative, but might account for some cases of progressive 'unmasking' of viral properties; (*b*) has not yet been proved for any specific case, but seems probably true of a number of viruses. The liberation of antigenic substances consisting of DNA nucleoproteins by healthy grafts and presumably by normal tissues, discovered by Medawar and his associates, and the probable liberation of specific growth 'antitemplates' by different tissues, indicated by Weiss, suggest ways in which an autogenous evolution of viruses could have occurred. In any case, investigation of tumour viruses and other paragenes is revealing many facts concerning non-nuclear and somatic replication, and illuminating the relations between genetics, development (epigenetics) and transmissibility (infection).

33. Epigenetics (Waddington's term for the analytical study of individual development) can shed light on the process of tumorigenesis, and *vice versa*.

34. Metaplasia is a purely descriptive term, denoting various

switches of differentiation (tissue transformation), including the irreversible transformation of undifferentiated cells of epidermis into mucous epithelium by excess vitamin A; of mesenchyme into bone or cartilage in mesenchymal tumours; the redifferentiation of tissues in regeneration after dedifferentiation to pluripotency; and the reversible transformations of cell-form in tissue-cultures. The alleged transformation of carcinomas to sarcomas is probably only apparent, and due to the acquisition of higher proliferation-rate and consequent greater visible dedifferentiation. Tumour tissue may exert an effect on the growth-rate of other tissues, both tumorous (as in mixed and composite tumours) and normal.

35. Dedifferentiation is a loose term comprising (*a*) reversal of determination (dedetermination, pluripotent dedifferentiation) as in regeneration blastemas; (*b*) loss of histological and functional differentiation, but without loss of primary differentiation, as in rapidly replicating tissues and those with high glycolytic metabolism (hypoplastic dedifferentiation); (*c*) anabiotic dedifferentiation, involving the reduction of general vital activities and entry into a resistant resting state, as in protozoan cysts and some tumour-cells subjected to deep freezing, especially when combined with dehydration and anaerobiosis. If such anabiotic dedifferentiation can be induced by anaerobiosis alone, it might account for the recurrence of operated cancers after long periods.

36. Tumorigenesis is not an abnormal type of differentiation, since (1) it may be reversible in early stages, (2) tumours retain their original determination, though in hypoplastic form, but depends on a metabolic deviation leading to breakdown of the normal mechanism controlling growth, and so to autonomy. It does not depend on escape from a local individuation field.

37. Tumorigenic metabolic deviation also differs from processes of normal differentiation in depending on a series of irreversible steps, and in the series not always being similar in different tissue culture strains, even of the same tissue. Each step is mutation-like in being a chance event and not the regular result of a statistical process, though differing from ordinary mutation in that multiple foci may sometimes be involved.

38. Berenblum distinguishes two main early steps in chemical carcinogenesis, a sudden *initiation* and a long latent period of

promotion: the latter can be induced by co-carcinogens which are incapable of inducing initiation. Nordling claims that at least six distinct steps are needed for a tissue to reach malignancy. Warburg also considers that a number of steps occur, but involving two main processes: (1) irreversible damage to the respiratory mechanism, followed by (2) a compensatory increase in glycolytic metabolism, both aerobic and anaerobic.

39. Malignancy is associated with a change in the surface properties of tumour cells, including electrical properties and lower calcium content, and leading to absence of adhesiveness.

40. Environmental carcinogenic agencies are of very various nature, including several types of radiation, many specific chemical compounds, certain types of prolonged irritation and certain parasites. Genetic carcinogenesis also occurs, due either to gross genetic imbalance in wide crosses or to single genes or systems of genes.

41. Some experimental carcinogens are also mutagenic; many inhibit growth, which might account for the long latent period in promotion. The action of some chemical carcinogens may be due to their combination with the nucleoprotein fibres of chromosomes. It has also been suggested that carcinogens may exert their effect by enzyme-deletion, by the progressive elimination of key proteins, or by damage to the cells' oxidative respiratory mechanism.

42. Endocrine-dependent tumours are caused by excess or defect of some normal endocrine activity, which may induce tumours in an endocrine-dependent tissue (e.g. mammary gland), or may upset an endocrine feed-back mechanism with resultant tumours in the same or another endocrine tissue.

43. In most tumorigenic processes there appear to be three main phases: (i) irreversible initiation and subsequent promotion (latent period); (ii) the conditioned phase—manifestation of a tumour but without irreversible autonomy; (iii) the fully autonomous phase. Before (i) we may prevent cancer but cannot (as yet) apply any treatment: after the onset of (iii) we cannot prevent but may treat it.

44. The chief methods of treatment, apart from operation, are (i) irradiation, (ii) serological (immunological), (iii) chemotherapeutic. The discovery of the distinctive surface properties of malignant cells may lead to more effective serological treatment. Chemotherapy may operate (*a*) by differential toxicity, (*b*) by interfering with DNA or

RNA synthesis by supplying analogues of nucleic acid components, and (*c*) by cytostatic agents.

45. Tumour regression (resorption) can best be understood as an effect of alteration in a metabolic (physiological) balance-system. Studies of the regression and resorption of denervated, reversed, ultraviolet-irradiated and xenoplastic urodele limbs, and of the various tissues during insectan and amphibian metamorphosis, should shed light on tumour regression. All larval tumours in *Drosophila*, and tumour grafts at some sites in amphibian larvae, regress at metamorphosis: Helff has caused regression of some tumours by extracts of metamorphosing amphibian tissues undergoing autolysis. Thyroxin appears to reduce the risk of recurrence of operated breast-cancer, presumably owing to a change in the body's metabolic balance.

46. The study of occupational, dietary, habitudinal, and other environmental cancers can point the way to measures of cancer prevention.

47. Cancer is a biological problem. The future organization of cancer research should include comparative studies, cytogenetics, epigenetics, allometric growth studies, and human ecology and demography, in addition to intensive biochemical analysis and the usual established disciplines of oncology.

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Note

The New York Academy of Sciences announced for October 25th, 1957, an important conference on the Genetic Concept for the Origin of Cancer.

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